Chapter 24
Metal Levels in Traditional Chinese and Ayurvedic Medicines

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Abstract The calcium, magnesium, nickel and zinc contents in seven Traditional Chinese Medicines (TCM) and four Ayurvedic Medicines (AM) purchased locally were determined using flame atomic absorption spectroscopy following five different digestion methods: four open wet hot plate with various mixtures of acids and/or H_2O_2, and microwave oven (**MWO**) with conc HNO_3 and H_2O_2. Sodium and potassium levels were analysed using flame photometry after **MWO** digestion. Potassium had the highest mean level among the six metals analysed. **MWO** was found to be more effective for Mg, method **M1** [conc HNO_3 + H_2O_2 (30 %)] for Ca and Zn, and method **M4** (aqua regia) for nickel. From replicate measurements, the inter-day precision of the flame photometric measurements for sodium and potassium was found to be 3.0–9.0 % and 1.3–9.0 % respectively. Comparison of results obtained from standard calibration curves and standard addition indicated that the percentage difference of sodium content in the TCM *Niuhuangchiehdu Pien* and the potassium content in AM, *Diabecon* was 3.9 % and 0.22 % respectively, indicating that the matrix did not have much influence on the metal content.

24.1 Introduction

The World Health Organisation (WHO) defines traditional medicine as: "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" [1].

There exist several traditional systems of medicine around the world, each with different associated philosophies and cultural origins, of which the two most

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prevalent are the Traditional Chinese Medicines (TCM) and Ayurvedic Medicines (AM). During the last two decades, there has been increased global interest in traditional medicines, especially those based on herbal products and extracts. These medicines contain a wide range of inorganic elements, both toxic and essential. Toxic metals of concern are mainly As, Cd, Hg and Pb. Several studies have reported potentially harmful levels of these heavy metals in both TCM and AM [2–24], thus emphasizing the need for more stringent quality standards and regulations for these products [8, 9, 21, 25, 26]. However, some authors highlighted that detailed chemical speciation be conducted to assess the effective toxicity of the medicines as the toxicity of elements varies with their chemical forms [3, 7, 10, 27].

On the other hand, TCM and AM are also known to have many essential and nutritional elements such as Ca, Cu, K, Mg, Mn, Na, Ni and Zn, whose excess or deficiency may disturb the normal biochemical functions of the human body. Several studies have reported elemental contents in traditional medicines or medicinal plant extracts [3, 11, 12, 17, 28–35].

Considering the importance of trace elements in various human metabolic processes and also their curative properties, the analysis of six essential and trace elements, namely calcium, magnesium, nickel, potassium, sodium and zinc, in 7 TCM and 4 AM commercially available in the Mauritian market was carried out.

Calcium is an essential plant nutrient and is omnipresent in all living things (animals and plants), water, soils and sediments. It is essential for healthy bones, teeth and blood. Deficiency of calcium causes rickets, osteomalacia and scurvy [36]. Magnesium is the fourth most abundant mineral in the human body and is needed for more than 300 biochemical reactions. It helps in maintaining a healthy immune system, strong bones and regulating the levels of several vitamins and minerals. Magnesium deficiency can cause amongst others cramps, muscle tension, poor memory, irritability, depression, and can result in low levels of calcium and potassium in the blood [37]. Nickel, despite being toxic, is found in minute amounts in the human body and is believed to play a role in the stabilisation of the RNA molecule, in the normal bone functioning, in the breakdown of glucose and human breast milk production. Deficiency of nickel is normally rare but can lead to liver, skin and kidney diseases. However, at high levels of accumulation, nickel is capable of causing serious health problems such as paralysis of the spinal column, tumours, thyroid and heart diseases [38]. Zinc is an essential element for the body and is involved in numerous aspects of cellular metabolism. Zinc plays important roles in normal growth and development during pregnancy, childhood and adolescence, and nearly 100 specific enzymes depend on zinc for their catalytic activity. Severe zinc deficiency can result in the slowing of growth and development, delayed sexual maturation, chronic and severe diarrhea, immune system deficiencies, impaired wound healing, reduced appetite, impaired taste sensation and night blindness [39]. Both potassium and sodium are essential body minerals. The body depends on the electrolytic properties of potassium for conduction of nerve impulses, muscle contractions and regulation of balance of acids and bases in the blood. Potassium is also involved in various biochemical processes in the human body. Some common problems associated with low potassium levels include hypertension, congestive heart failure, cardiac arrhythmia, fatigue and

osteoporosis [40]. On the other hand, the body uses sodium to regulate blood pressure and blood volume. Sodium is also essential for the transmission of nerve impulses, heart activity, and certain metabolic functions. Low sodium levels (hyponatremia) are uncommon but can occur whenever there is an increase in the amount of body water relative to sodium. On the contrary, hypernatremia (high sodium levels) is common, caused principally with high sodium content in food. Other causes include kidney diseases, low water intake, severe diarrhea and vomiting [41].

24.2 Methodology

24.2.1 Materials and Reagents

7 TCM (*Jin Qian Chao, Leung Pui Kee, Po Chai Pills, Quiang Li Tien-Ma, Saplingtan, Watermelon Frost* and *Niuhuangchiehdu Pien*) imported from China and 4 AM (*Diabecon, Pilex, Septilin* and *Softovac*) imported from India were purchased locally.

The standard stock 1,000 ppm solutions of potassium and sodium were bought from Sherwood Scientific Ltd, calcium and zinc from Fisher Scientific, magnesium and nickel from Fisons Scientific. Concentrated HNO_3, HCl and H_2SO_4 were obtained from SD Fine Chemicals Ltd while H_2O_2 (30 %) from Fisons Scientific.

All glassware used (burettes, pipettes, volumetric flasks, beakers) were of grade A. Prior to use, all glassware were acid-washed.

24.2.2 Sample Preparation

The traditional medicines which were mostly in the form of pills were ground using a mortar and pestle in order to obtain fine and homogeneous solids.

24.2.3 Microwave Oven Digestion

The samples were digested using the microwave digester Milestone High Performance Microwave Unit mls 1,200 M. Prior to use, the Teflon flasks were acid washed. Approximately 1 g of each powdered sample was weighed in duplicate in dry labelled Teflon flasks to which were added 7 ml of concentrated nitric acid (72 %) followed by 5 ml of H_2O_2 (30 %). The samples were digested using the program shown in Table 24.1.

After cooling for about 30 min, the resulting digested samples were filtered through fluted filter paper into 25 mL volumetric flasks and the filtrates were made up to the mark with distilled deionised water. The resulting clear solutions were then transferred to clean acid-washed labelled plastic bottles.

Table 24.1 Microwave digestion program used

Step	1	2	3	4	5
Power/W	250	0	250	400	650
Time/min	1	1	5	5	5

Table 24.2 Reagents used for wet open digestions

Method	Reagents
M1	HNO_3 (72 %): 16 ml & H_2O_2 (30 %): 4 ml
M2	HNO_3 (72 %): 20 ml
M3	HNO_3 (72 %): 16 ml & H_2SO_4 (98 %): 4 ml
M4	HCl (35 %): 15 ml & HNO_3 (72 %): 5 ml

Table 24.3 Concentration of standards and wavelengths used

Metal	Wavelength/nm	Concentration of prepared standards/ppm
Calcium	422.7	1, 2, 3, 4 and 5
Magnesium	202.6	5, 10, 15, 20 and 25
Nickel	232.0	2, 4, 6, 8 and 10
Zinc	213.9	0.2, 0.4, 0.8, 1.0 and 1.2
Potassium	766.0	10, 15, 20, 25 and 30
Sodium	589.0	10, 15, 20, 25 and 30

24.2.4 Wet Open Digestions

Wet open digestion of the samples was performed using four different methods as given in Table 24.2.

Approximately 1 g of the ground medicine was weighed in duplicate into separate 50 mL conical flasks. The appropriate reagents as detailed in Table 24.2 were pipetted into each of the flasks. The solutions were heated to boiling on a hot plate in a fume hood for 4 h before filtration into 25 mL volumetric flasks and making up to the mark with distilled deionised water. The contents were finally transferred to clean well-labelled plastic bottles.

Reagent blanks were prepared for both microwave and wet open digestions under similar conditions as those used for the medicinal samples.

24.2.5 Determination of Metal Contents

The standard calibration solutions were prepared daily from the stock solutions by proper dilution with distilled deionised water. The levels of potassium and sodium in the medicines were determined using a CIBA Corning Model 410 flame photometer whereas calcium, magnesium, nickel and zinc contents were analysed by a GBC Avanta flame atomic absorption spectrometer using air/acetylene. Table 24.3 lists the concentrations of the prepared standard solutions and wavelength used for the metals.

24.2.6 Standard Addition

Determination of Potassium Content in *Diabecon*

5 ml of the microwave oven digested medicinal sample *Diabecon* was diluted 20 times. 5 ml of this diluted sample was then transferred to each of five 25 mL volumetric flasks. To the five volumetric flasks, 0, 0.5, 1, 1.5 and 2 ml of 100 ppm potassium standard solution was respectively added. The resultant solutions were then made up to the mark with distilled deionised water and analysed by flame photometry. The concentration of the added standard for each flask was determined and a plot of emission against concentration of added standard was drawn. The concentration of potassium in the diluted sample corresponds to the x-intercept obtained by extrapolation. The concentration of potassium in the undiluted sample is then calculated by taking into consideration the dilution performed.

Determination of Sodium Content in *Niuhuangchiehdu Pien*

The experimental procedure is similar to that applied in section "Determination of Potassium Content in *Diabecon*" except that 5 ml of the undiluted microwave digested *Niuhuangchiehdu Pien* sample was pipetted in the five volumetric flasks.

24.3 Results and Discussion

24.3.1 Metal Levels in TCM and AM

Calcium

Table 24.4 gives the calcium levels obtained in the 7 TCM and 4 AM following the five methods of digestion.

All the 11 medicinal samples analysed exhibited detectable calcium levels. The highest calcium level is observed in *Niuhuangchiehdu Pien* irrespective of the applied method of digestion. The elevated concentration of calcium found in *Niuhuangchiehdu Pien* can be accounted by the presence of the gypsum selenite (26.64 %) in its formulation. Excluding the TCM *Niuhuangchiehdu Pien*, the calcium content in the four AM is higher than in the remaining six TCM. *Diabecon*, *Pilex* and *Softovac* have the highest amount of calcium amongst the AM. However, the calcium contents were considerably lower than those analysed by Giacomino et al. [12] in five products of the Parpati family where Ca was present in all the medicines with a range of 738–8,439 μg/g. Two main ingredients namely *Shilajit* and *Pterocarpus marsupium* present in *Diabecon* contain high calcium levels. *Shilajit* is an exudate that is pressed out from rock layers in sacred mountains in

Table 24.4 Calcium levels (μg/g) in the 7 TCM and 4 AM following the five methods of digestion

Medicine	**M1**	**M2**	**M3**	**M4**	**MWO**
Diabecon	129 ± 2	117 ± 2	93 ± 2	108 ± 6	116 ± 9
Pilex	132 ± 2	109 ± 0	44 ± 2	59 ± 6	120 ± 2
Septilin	70 ± 4	40 ± 4	53 ± 2	26 ± 6	53 ± 2
Softovac	131 ± 0	65 ± 11	47 ± 2	70 ± 4	113 ± 0
Jin Qian Chao	59 ± 2	41 ± 6	33 ± 4	40 ± 4	41 ± 6
Leung Pui Kee	76 ± 4	36 ± 4	21 ± 4	27 ± 4	53 ± 6
Po Chai Pills	67 ± 4	21 ± 4	17 ± 2	20 ± 2	56 ± 2
Quiang Li Tien-Ma	36 ± 4	32 ± 2	26 ± 2	24 ± 9	33 ± 4
Saplingtan	78 ± 2	67 ± 9	43 ± 4	46 ± 0	65 ± 2
Watermelon Frost	59 ± 2	32 ± 2	52 ± 0	50 ± 2	52 ± 0
Niuhuangchiehdu Pien	865 ± 2	912 ± 4	856 ± 19	706 ± 9	876 ± 4

Nepal and other high mountains and is rich in minerals and metals such as calcium, zinc, sodium and magnesium [42]. On the other hand, *Pterocarpus marsupium* also known as the Indian Kino tree contains 10–20 % of calcium sennoside which is a big molecule with molecular formula $C_{42}H_{36}CaO_{20}$.

Comparison between the various digestion methods showed that **M1** digestion resulted in the highest calcium levels for 10 out of the 11 medicinal samples analysed in this study, except for the TCM *Niuhuangchiehdu Pien* in which the highest calcium content was detected following digestion method **M2**. **MWO** digestion gave the next highest calcium levels for the medicines analysed. Least calcium level was observed in seven medicines (*Diabecon*, *Pile*x, *Softovac*, *Jin Qian Chao*, *Leung Pui Kee*, *Po Chai Pills* and *Saplingtan*) following digestion method **M3**, in three medicines (*Septilin*, *Quiang Li Tien-Mo* and *Niuhuangchiehdu Pien*) from digestion method **M4** and in one medicine (*Watermelon Frost*) from digestion method **M2**.

The statistical two-tail student's *t*-test method was applied to compare the different digestion methods to investigate whether there are any significant differences between the results obtained from all the ten possible pairs (Table 24.5). By analysing the calculated *t*-values (t_{calc}) with the t_{crit} value (2.23), it is observed that for six pairs of digestion methods, namely **M1**/**M2**, **M1**/**M3**, **M1**/**M4**, **M1**/**MWO**, **M3**/**MWO** and **M4**/**MWO**, the results are significantly different from each other at 95 % confidence level. The results obtained following the digestion method **M1** are thus significantly different from those obtained from the other four digestion methods. Similarly, calcium levels obtained following **MWO** digestion are significantly different from all the other digestion methods except **M2**. On the other hand, it can be concluded that the values obtained from digestion methods **M2**, **M3** and **M4** are comparable and hence are not significantly different.

Magnesium

The magnesium levels obtained in the 7 TCM and 4 AM following the five methods of digestion are shown in Table 24.6.

Table 24.5 Comparison of calcium content between different pairs of digestion methods using *t*-test

Digestion pair	Mean difference	Standard deviation	t_{calc}
M1/M2	20.91	28.69	2.42
M1/M3	37.91	28.69	4.38
M1/M4	47.82	42.16	3.67
M1/MWO	11.27	9.22	4.05
M2/M3	17.00	25.63	2.20
M2/M4	26.91	61.79	1.44
M2/MWO	9.64	21.88	1.46
M3/M4	9.91	48.21	0.68
M3/MWO	26.64	25.30	3.49
M4/MWO	36.55	47.95	2.53

Table 24.6 Magnesium levels (μg/g) in the 7 TCM and 4 AM following the five methods of digestion

Medicine	**M1**	**M2**	**M3**	**M4**	**MWO**
Diabecon	97 ± 4	80 ± 1	81 ± 2	83 ± 7	99 ± 2
Pilex	258 ± 2	230 ± 2	237 ± 2	242 ± 4	265 ± 2
Septilin	76 ± 1	57 ± 5	64 ± 2	67 ± 2	78 ± 2
Softovac	52 ± 1	42 ± 1	48 ± 1	48 ± 1	55 ± 4
Jin Qian Chao	128 ± 1	119 ± 2	125 ± 1	123 ± 5	137 ± 9
Leung Pui Kee	64 ± 2	48 ± 1	59 ± 4	57 ± 2	62 ± 1
Po Chai Pills	61 ± 2	48 ± 4	54 ± 2	57 ± 2	62 ± 4
Quiang Li Tien-Ma	71 ± 2	50 ± 2	61 ± 2	64 ± 2	76 ± 2
Saplingtan	42 ± 1	23 ± 2	26 ± 2	31 ± 1	42 ± 1
Watermelon Frost	36 ± 2	23 ± 2	28 ± 4	26 ± 2	38 ± 4
Niuhuangchiehdu Pien	71 ± 2	57 ± 2	62 ± 1	69 ± 4	74 ± 2

All the traditional medicines analysed were found to contain magnesium. The highest magnesium level was found in the AM *Pilex* which is due to the presence of the ingredients *Guggul* and *Shilajit*. *Guggul* is composed of a mixture of organic compounds, inorganic ions and minerals which consist mostly of calcium, magnesium and iron [43]. On the other hand, *Shilajit* is a rich source of naturally occurring minerals like magnesium [42]. The lowest magnesium level in the AM was found in *Softovac*. For the TCM, *Jin Qian Chao* was found to have the highest magnesium content. Magnesium is well-known for its calcium channel blocking effect and can reduce the formation of calcium oxalate which is the main cause for stones in body. *Herba Lysmachiae*, the only ingredient of *Jin Qian Chao*, is very effective in dissolving kidney, liver and gall stones [44] and this can explain the high level of magnesium obtained in this TCM. The range of magnesium levels obtained in this study for AM corresponds to only a certain extent to the range observed by Giacomino et al. [12] (201–4,236 μg/g). For the TCM, the range observed was within that obtained by Wang et al. [11] (ND-2,031 μg/g). The lowest magnesium levels were found in the TCM *Saplingtan* and *Watermelon Frost*.

Highest levels of magnesium were observed after **MWO** or **M1** digestion for all the 11 medicines. Digestion method **M2** gave the least amount for all the medicines

Table 24.7 Comparison of magnesium content between different pairs of digestion methods using *t*-test

Digestion pair	Mean difference	Standard deviation	t_{calc}
M1/M2	16.27	5.42	9.95
M1/M3	10.09	5.66	5.91
M1/M4	8.09	4.39	6.11
M1/MWO	2.91	3.11	3.10
M2/M3	6.18	2.96	6.93
M2/M4	8.18	3.79	7.16
M2/MWO	19.18	6.45	9.87
M3/M4	2.00	3.13	2.12
M3/MWO	13.00	6.60	6.53
M4/MWO	11.00	5.51	6.62

Table 24.8 Nickel levels (μg/g) in the 7 TCM and 4 AM following the five methods of digestion

Medicine	**M1**	**M2**	**M3**	**M4**	**MWO**
Diabecon	7.3 ± 0.3	7.0 ± 0.6	6.7 ± 0.3	7.7 ± 0.6	8.6 ± 0.3
Pilex	5.4 ± 0.3	5.4 ± 0.3	5.7 ± 0.6	6.1 ± 0.9	7.0 ± 0.6
Septilin	5.4 ± 0.3	6.1 ± 0.3	5.7 ± 0.1	6.7 ± 0.3	6.4 ± 0.6
Softovac	4.5 ± 0.6	4.8 ± 0.3	5.4 ± 0.9	6.1 ± 0.3	6.1 ± 0.3
Jin Qian Chao	6.7 ± 0.9	7.0 ± 0.6	7.6 ± 1.3	8.9 ± 0.6	8.6 ± 0.3
Leung Pui Kee	7.3 ± 0.3	8.6 ± 0.3	7.7 ± 0.6	13.1 ± 0.9	8.9 ± 1.3
Po Chai Pills	7.7 ± 0.6	8.3 ± 0.6	8.9 ± 0.6	8.9 ± 0.1	11.2 ± 0.3
Quiang Li Tien-Ma	7.7 ± 0.1	6.1 ± 0.3	6.1 ± 0.3	9.2 ± 0.3	9.2 ± 0.9
Saplingtan	3.2 ± 0.6	5.1 ± 0.6	5.7 ± 0.1	8.6 ± 0.3	7.0 ± 0.1
Watermelon Frost	3.5 ± 0.3	4.5 ± 0.1	4.8 ± 0.3	8.6 ± 0.3	8.3 ± 0.6
Niuhuangchiehdu Pien	4.5 ± 0.6	6.4 ± 0.6	7.7 ± 0.1	8.3 ± 0.6	8.0 ± 0.3

analysed. The following order of extractability of magnesium was observed: **M2** $<$ **M3** ~ **M4** $<$ **M1** ~ **MWO**. From Table 24.7, it was observed that the t_{calc} was greater than the t_{crit} (2.23) for all pairs of digestion methods except **M3/M4**.

Nickel

Table 24.8 gives the nickel levels obtained in the 7 TCM and 4 AM following the five methods of digestion.

Nickel was detected in all the 11 medicines. Highest level was observed in the TCM *Leung Pui Kee*. The latter is based mainly on constituents of plant origin and the only mineral present is talc (1 %). A potential source of nickel apart from talc is soils and sediments. Plants grown in soils rich in nickel absorb the metal which is then incorporated into the medicine. The plant *Aster tataricus* used in the preparation of *Leung Pui Kee* is native from the northern part of China and is grown in meadows and wetlands which are rich in minerals and metals due to the anthropogenic activities in the vicinity. The presence of nickel in the other medicines can also be explained from soil contribution.

Table 24.9 Comparison of nickel content between different pairs of digestion methods using *t*-test

Digestion pair	Mean difference	Standard deviation	t_{calc}
M1/M2	0.59	0.31	1.89
M1/M3	0.85	0.41	2.09
M1/M4	2.67	0.62	4.27
M1/MWO	2.34	0.44	5.36
M2/M3	0.26	0.19	1.40
M2/M4	2.08	0.45	4.60
M2/MWO	1.74	0.37	4.65
M3/M4	1.82	0.52	3.46
M3/MWO	1.48	0.33	4.47
M4/MWO	0.33	0.56	0.60

The nickel levels in the 11 medicines were all found in a small range (3.2–13.1 μg/g) and are comparable to those observed by Wieckowski and Mezyk [45] in TCM (1.6–11.3 μg/g), and by Akhtar et al. [46] (0.94–4.35 μg/g) in AM.

Comparison between the five different methods of digestion for nickel revealed that methods **M4** and **MWO** were the best acid mixtures for the medicinal samples analysed in this study. Highest deviations between the digestion methods **MWO** and **M4** were observed for *Leung Pui Kee* (−46.4 %), *Saplingtan* (−22.8 %) and *Po Chai Pills* (+20.0 %). The two-tail student's *t*-test was applied to compare the results obtained from all the different pairs of digestion methods and the results are shown in Table 24.9.

It can be observed that for six out of ten combinations analysed, the t_{calc} is greater than the t_{crit} (2.23). Results following digestion methods **M4** and **MWO** are thus significantly different at the 95 % confidence level from those obtained after digestion methods **M1**, **M2** and **M3**. On the other hand, no significant difference was observed between the three methods of digestion **M1**, **M2** and **M3** and between **M4** and **MWO** indicating that the values obtained were comparable.

Zinc

The zinc levels obtained in the 7 TCM and 4 AM following the five methods of digestion are given in Table 24.10.

Zinc was detected in all the 11 traditional medicines analysed. The highest zinc level observed was in *Po Chai Pills* for the TCM and in *Diabecon* for the AM. The zinc level in the TCM *Po Chai Pills* can be explained by the presence of the ingredient *Cortex Magnolia Officinalis* which is rich in a number of trace elements including zinc [47]. On the other hand, the zinc content in *Diabecon* is mainly due to two of its constituents, namely *Yashad Bhasma* and *Shilajit*. *Yashad Bhasma* also known as zinc calx, is processed zinc which is obtained from the calcination of *Rasaka satva* which is a metallic extract of zinc carbonate/zinc oxide [48]. On the other hand, *Shilajit* is a rich source of essential and trace minerals including zinc [42]. The range for zinc in the TCM was found to be within that obtained by Shao

Table 24.10 Zinc levels (μg/g) in the 7 TCM and 4 AM following the five methods of digestion

Medicine	**M1**	**M2**	**M3**	**M4**	**MWO**
Diabecon	26.8 ± 0.2	24.0 ± 0.3	22.3 ± 0.2	25.5 ± 0.1	22.0 ± 0.1
Pilex	11.8 ± 0.1	8.7 ± 0.6	11.1 ± 0.4	9.7 ± 0.2	9.9 ± 0.1
Septilin	13.3 ± 0.1	10.7 ± 0.4	12.9 ± 0.1	9.2 ± 0.1	10.1 ± 0.2
Softovac	11.8 ± 0.1	8.2 ± 0.2	11.6 ± 0.1	6.3 ± 0.2	9.7 ± 0.2
Jin Qian Chao	9.6 ± 0.1	7.9 ± 0.1	9.4 ± 0.1	5.1 ± 0.2	6.5 ± 0.2
Leung Pui Kee	16.9 ± 0.1	14.2 ± 0.2	16.2 ± 0.1	13.8 ± 0.1	15.0 ± 0.3
Po Chai Pills	26.7 ± 0.1	25.0 ± 0.3	26.4 ± 0.2	18.8 ± 0.2	22.7 ± 0.1
Quiang Li Tien- Ma	13.5 ± 0.1	12.0 ± 0.2	13.1 ± 0.1	10.6 ± 0.1	11.9 ± 0.1
Saplingtan	3.7 ± 0.1	3.2 ± 0.1	3.5 ± 0.1	3.2 ± 0.1	2.8 ± 0.2
Watermelon Frost	4.5 ± 0.1	3.9 ± 0.2	4.0 ± 0.1	3.6 ± 0.1	4.0 ± 0.1
Niuhuang chiehdu Pien	8.1 ± 0.3	6.0 ± 0.2	4.0 ± 0.3	4.4 ± 0.2	5.1 ± 0.2

et al. [49] (3.2–86.2 μg/g). Similarly, the range of zinc found in the AM is within the range (4–109 μg/g) observed by Al-Omari [17].

The wet digestion **M1** using HNO_3 and H_2O_2 was found to result in the highest zinc levels followed by **M3**. Digestion method **M4** gave the least zinc level for seven out of the eleven medicines analysed. Using *t*-test, it was found that the t_{calc} was greater than the t_{crit} (2.23) at 95 % confidence for the following digestion pairs: **M1/M2**, **M1/M3**, **M1/M4**, **M1/MWO, M3/M4** and **M3/MWO** (Table 24.11), inferring that there is a significant difference between the results obtained after **M1** digestion method and the other digestion methods (**M2**, **M3**, **M4** and **MWO**).

Potassium and Sodium

Table 24.12 gives the potassium and sodium levels obtained in the 7 TCM and 4 AM by flame photometry following **MWO** digestion.

The ranges observed for potassium were 6,200–21,143 μg/g and 31.7–26,416 μg/g for the AM and TCM respectively. The highest potassium content was found in the TCM *Jin Qian Chao* (26,416 μg/g) followed by the AM *Diabecon* (21,143 μg/g). As *Jin Qian Chao* is composed solely of *Herba Lysimachiae*, the high potassium content of the TCM may be due to the presence of inorganic potassium chloride absorbed from the soil in the herb [44]. On the other hand, the high amount of potassium in *Diabecon* results from the ingredients *Shilajit* and *Abhrak Bhasma*. *Shilajit* is obtained from the Himalayan Mountains and is composed of a large number of essential metals in the ionic form including potassium [42]. *Bhasmas* are prepared by calcination of metals and *Abhrak Bhasma* contains trace amounts of potassium [48]. The highest level in the TCM observed by Wang et al. [11] was 11,054 μg/g which is lower than that observed in the present study (26,416 μg/g). The lowest potassium contents were observed in two TCM, namely *Saplingtan* (31.7 μg/g) and *Watermelon Frost* (70.5 μg/g). On the other hand, the range of potassium levels observed in the AM was within that obtained by Giacomino et al. [12] (44.5–21,440 μg/g).

Table 24.11 Comparison of zinc content between different pairs of digestion methods using *t*-test

Digestion pair	Mean difference	Standard deviation	t_{calc}
M1/M2	2.08	0.99	6.96
M1/M3	1.11	1.59	2.31
M1/M4	3.32	2.18	5.05
M1/MWO	2.45	1.30	6.27
M2/M3	0.97	1.68	1.92
M2/M4	1.24	2.08	1.97
M2/MWO	0.37	1.24	0.99
M3/M4	2.21	2.98	2.46
M3/MWO	1.34	1.40	3.18
M4/MWO	0.86	1.94	1.48

Table 24.12 Potassium and sodium levels (μg/g) in the 7 TCM and 4 AM after **MWO** digestion

Medicine	Potassium	Sodium
Diabecon	21,143 ± 7	5,173 ± 73
Pilex	6,408 ± 71	1,972 ± 73
Septilin	6,200 ± 3	3,500 ± 1
Softovac	6,690 ± 352	1,460 ± 2
Jin Qian Chao	26,416 ± 338	3,350 ± 4
Leung Pui Kee	9,795 ± 68	1,535 ± 73
Po Chai Pills	7,677 ± 71	1,099 ± 73
Quiang Li Tien-Ma	5,844 ± 71	1,608 ± 146
Saplingtan	31.7 ±3.5	ND
Watermelon Frost	70.5 ± 0.1	6,191 ± 73
Niuhuangchiehdu Pien	4,575 ± 71	1,099 ± 73

The sodium levels observed ranged from 1,099 μg/g in *Po Chai Pills* and *Niuhuangchiehdu Pien* to 6,191 μg/g in *Watermelon Frost*. In *Saplingtan*, the sodium level was below the detection limit of the apparatus. The highest sodium level was observed in *Watermelon Frost* (6,191 μg/g) which is used for the treatment of throat diseases, general wounds with bleeding, toothache, burns and scald, and can be accounted by the presence of the ingredient Mirabilite (also known as Glauber's salt, $Na_2SO_4{\cdot}10H_2O$) in its formulation. Due to its anti-bacterial property, sodium is also present in high amount in *Septilin* (3,500 μg/g) which is mainly used as an anti-infective therapy for the treatment of sinusitis. The high sodium level observed (5,173 μg/g) in *Diabecon* can be accounted by the presence of *Shilajit*, a major ingredient rich in various metallic salts including sodium [42].

To investigate the matrix effect in the medicines, the potassium level in the AM, *Diabecon* and the sodium level in the TCM *Niuhuangchiehdu Pien*, were determined by the method of standard addition and the results obtained compared with those obtained from standard calibration curves. The plots obtained for potassium level in *Diabecon* and sodium level in *Niuhuangchiehdu Pien* are depicted in Figs. 24.1 and 24.2 respectively.

The potassium and sodium levels obtained from both standard calibration curve and standard addition are shown in Table 24.13.

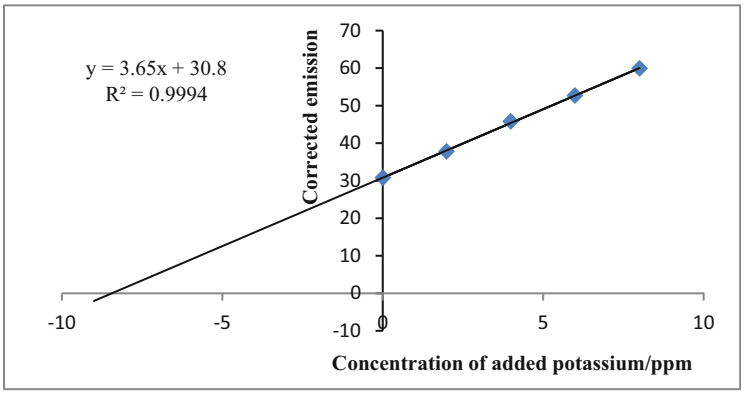

Fig. 24.1 Plot of corrected emission against concentration of added potassium for *Diabecon*

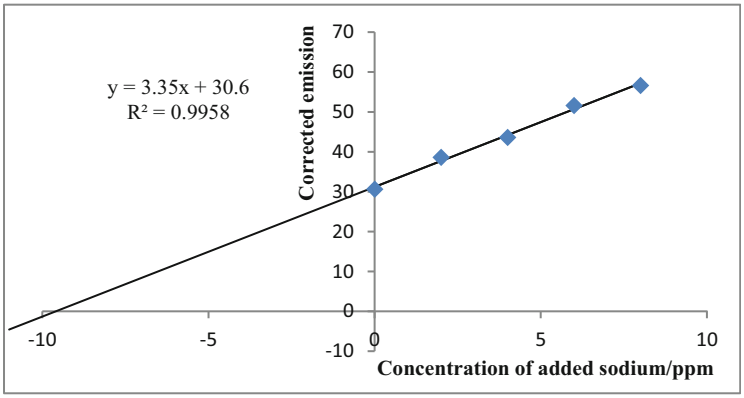

Fig. 24.2 Plot of corrected emission against concentration of added sodium for TCM *Niuhuangchiehdu Pien*

It is observed that the sodium and potassium concentration obtained from standard addition are close to those obtained from standard calibration curves with a CV of 0.22–3.9 %. It can thus be deduced that the matrix did not have much influence on the sodium or potassium content.

24.3.2 Precision of Flame Photometric Method

In this study, intra-day precision, also known as repeatability, was determined for the potassium content in the TCM *Po Chai Pills* and inter-day precision for the potassium and sodium content for all the medicines. The precision of an analytical procedure is usually expressed as the % relative standard deviation (%RSD) of a

Table 24.13 Concentrations obtained by standard calibration curve and standard addition

	Potassium level (μg/g)			Sodium level (μg/g)		
Medicine	Calib curve	Std add	CV (%)	Calib curve	Std add	CV (%)
Diabecon	21,143	21,096	0.22	–	–	–
Niuhuangchiehdu Pien	–	–	–	1,099	1,142	3.9

Table 24.14 Intra-day precision data

Time/min	Potassium level (μg/g)
0	7,610
15	7,469
30	7,610
45	7,469
60	7,469
75	7,469
90	7,469
105	7,469
120	7,469
135	7,610
Mean ± SD	7,511 ± 68
%RSD	0.91

series of measurements. Precision is concentration dependent and the acceptable precision (%RSD) for concentration (≥ 100 ppm) is 15 % [50].

Intra-Day Precision

Intra-day precision expresses the precision under the same operating conditions over a short interval of time within the same day and was determined for the potassium content by flame photometry in the TCM *Po Chai Pills* by measuring the same sample ten times at 15 min' time interval. The results are shown in Table 24.14.

The repeatability of the analytical method for the TCM *Po Chai Pills* was excellent as the %RSD was found to be 0.91 %.

Inter-Day Precision

The inter-day precision of the photometric analyses and the method validity were assessed by analysing the microwave-digested samples of the 11 medicines on five different days: day 1, day 2, day 3, day 8 and day 15 for potassium and sodium. The results are shown in Table 24.15 and Fig. 24.3 respectively.

It was observed that the %RSD for all the medicines (1.3–9.0 %) for both the potassium and sodium analysis were well below the acceptable precision and thus it can be inferred that the inter-day precision for the analytical method was good.

Table 24.15 Inter-day precision analysis of potassium

	Potassium level (μg/g)						
Medicine	Day 1	Day 2	Day 3	Day 8	Day 15	Mean ± SD (μg/g)	%RSD
Diabecon	21,143	21,485	20,931	21,010	21,675	21,249 ± 285	1.3
Pilex	6,408	6,607	6,518	6,728	6,497	6,552 ± 109	1.7
Septilin	6,197	6,675	6,234	6,293	6,136	6,307 ± 191	3.0
Softovac	6,690	6,948	6,589	6,800	6,786	6,763 ± 120	1.8
Jin Qian Chao	26,416	27,628	25,191	26,448	28,541	26,845 ± 1,146	4.3
Leung Pui Kee	9,795	10,156	9,784	9,990	9,967	9,938 ± 138	1.4
Po Chai Pills	7,677	7,972	7,441	7,743	7,654	7,697 ± 171	2.2
Quiang Li Tien Ma	5,844	5,924	5,595	5,858	6,208	5,886 ± 196	3.3
Saplingtan	32	27.3	28.4	25.4	25.3	27.7 ± 2.5	9.0
Watermelon Frost	71	61	61	61	54.2	61.6 ± 5.4	8.8
Niuhuangchieh du Pien	4,575	4,423	4,175	4,274	4,835	4,456 ± 233	5.2

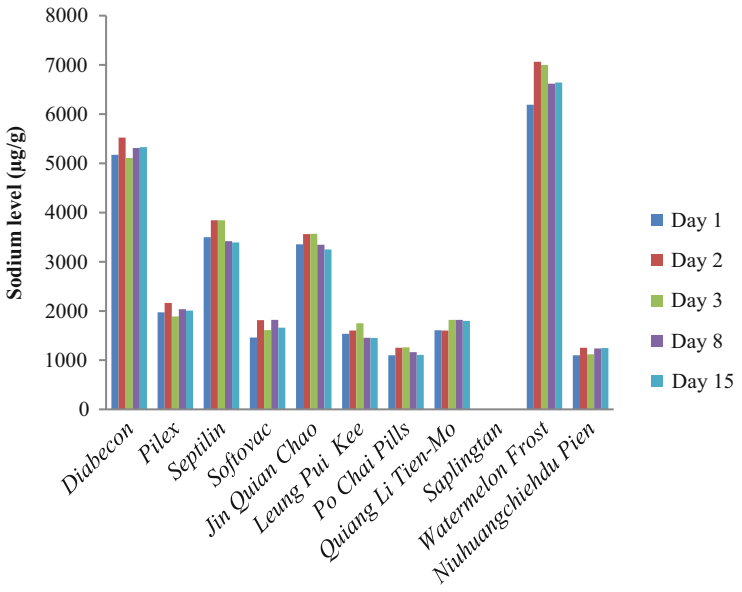

Fig. 24.3 Variation of the sodium content of the TCM and AM on an inter-day basis

24.4 Conclusions

In this study, 7 TCM and 4 AM purchased in Mauritius were analysed for their calcium, magnesium, nickel and zinc levels by flame atomic absorption spectroscopy after digestion by four wet open methods and **MWO**. The results obtained after the different methods of digestion were compared using the student's *t*-test.

In addition, the potassium and sodium levels in the 11 medicines were determined by flame photometry, whose validity was checked by analyzing its precision.

In both the TCM and AM, potassium was present in the highest amount. The highest calcium, magnesium, nickel, potassium, sodium and zinc level were observed respectively in *Niuhuangchiehdu Pien* (912 μg/g), Pilex (265 μg/g), *Leung Pui Kee* (13.1 μg/g), *Jin Qian Chao* (26,416 μg/g), *Watermelon Frost* (6,191 μg/g) and *Diabecon* (26.8 μg/g). **MWO** digestion was found to be more effective for Mg, method **M1** [conc HNO_3 + H_2O_2 (30 %)] for Ca and Zn, and method **M4** (aqua regia) for nickel.

From replicate measurements, the precision of the flame photometric method used for the analysis of potassium was found to be good as the %RSD for both intra-day (0.91 %) and inter-day (1.3–9.0 %) precision was less than the acceptable % RSD (15 %). For sodium, the inter-day precision was found to be in the range 3.0–9.0 %.

To investigate matrix interference, standard addition was carried out on the AM *Diabecon* for potassium and the TCM *Niuhuangchiehdu Pien* for sodium. A coefficient of variation of 0.22 % and 3.9 % was observed for the potassium and sodium level respectively indicating little interference from the sample matrix.

Further study is under way for the analysis of other metals, in particular toxic ones in these TCM and AM.

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