Chapter 6

Quantitation of Haloperidol, Fluphenazine, Perphenazine, and Thiothixene in Serum or Plasma Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Abstract

Haloperidol, fluphenazine, perphenazine, and thiothixene are "typical" antipsychotic drugs that are used in the treatment of schizophrenia and other psychiatric disorders. The monitoring of the use of these drugs has applications in therapeutic drug monitoring and overdose situations. LC-MS/MS is used to analyze plasma/serum extracts with deuterated analog of imipramine as the internal standard to ensure accurate quantitation and control for any potential matrix effects. Positive ion electrospray is used to introduce the analytes into the mass spectrometer. Selected reaction monitoring of two product ions for each analyte allows for the calculation of ion ratios which ensures correct identification of each analyte, while a matrix-matched calibration curve is used for quantitation.

Key words Haloperidol, Fluphenazine, Perphenazine, Thiothixene, Plasma, Serum, UPLC, Mass spectrometry

1 Introduction

Fluphenazine (e.g. Prolixin) and perphenazine (e.g. Etrafon) are phenothiazine neuroleptics used in the management of psychotic disorders, such as schizophrenia, mania, anxiety/agitation, and depression. Haloperidol (e.g. Haldol) is a butyrophenone typical antipsychotic drug indicated for use in the treatment of schizophrenia and the control of tics and vocal utterances of Tourette's disorder in children and adults. Thiothixene (e.g. Navane) is a thioxanthene neuroleptic with general properties similar to those of the phenothiazines. Therapeutic monitoring of concentrations of these drugs is useful in optimizing therapy, evaluate compliance, and to monitor for adverse drug reaction [1, 2].

This chapter describes an analytical method to measure the above-mentioned four typical antipsychotic drugs in human serum/plasma by precipitating serum/plasma proteins and

Uttam Garg (ed.), *Clinical Applications of Mass Spectrometry in Drug Analysis: Methods and Protocols*, Methods in Molecular Biology, vol. 1383, DOI 10.1007/978-1-4939-3252-8_6,

collecting the supernatant for analysis. The supernatant is injected onto the LC-MS/MS. Qualitative identification is made using unique MS/MS transitions, ion ratios of those transitions, and chromatographic retention time. Quantitation is performed using a daily calibration curve of prepared calibration samples and using peak area ratios of analyte to internal standard to establish the calibration model. Patient sample concentrations are calculated based on the calibration model's mathematical equation. Quantitative accuracy is monitored with QC samples independently prepared with known concentrations of analyte and comparing the calculated concentration with the expected concentration [3, 4].

2 Materials

2.1 Samples

1. Pre-dose (trough) draw—at steady-state concentration for serum/plasma. Separate serum or plasma from cells within 2 h of collection.
2. Collect in plain red tube. Avoid gel or other separator tubes.
3. Specimens can be stored for at least 24 h ambient, 5 days refrigerated, 30 days frozen prior to analysis.

2.2 Reagents

1. Clinical Laboratory Reagent Water (CLRW).
2. Verified negative serum/plasma pool.
3. Mobile Phase A (CLRW with 0.1 % Formic Acid): 1.0 mL of concentrated Formic Acid in CLRW q.s. to 1.0 L in volumetric flask.
4. Mobile Phase B (Acetonitrile with 0.1 % Formic Acid): 1.0 mL of concentrated Formic Acid in LC-MS grade Acetonitrile q.s. to 1.0 L in volumetric flask.

2.3 Standards and Calibrators

1. Haloperidol, 1.0 mg/mL stock standard prepared in Methanol (Cerilliant, Round Rock, TX).
2. Perphenazine 1.0 mg/mL stock standard prepared in Methanol (Cerilliant, Round Rock, TX).
3. Fluphenazine 1.0 mg/mL stock standard prepared in Methanol (Cerilliant, Round Rock, TX).
4. Thiothixene 1.0 mg/mL stock standard prepared in Methanol (Cerilliant, Round Rock, TX).
5. Prepare an intermediate solution containing fluphenazine and perphenazine at 1000 ng/mL and haloperidol and thiothixene at 5000 ng/mL in methanol. Add ~3 mL of methanol to a 10 mL volumetric flask. Add 10 µL each of fluphenazine and perphenazine reference materials and 50 µL each of haloperidol and thiothixene reference material to the flask, q.s. to

Table 1
Preparation of calibrators. The total volume is made to 25 mL with drug-free human serum/plasma

Calibrator	Volume of intermediate solution (μL)	Final [], ng/mL
1	5	0.2/1[a]
2	50	2/10[a]
3	100	4/20[a]
4	300	12/60[a]

[a]Fluphenazine and perphenazine/haloperidol and thiothixene

10 mL with methanol, add a stir bar and stopper and mix for 30 min at room temperature. Aliquot as appropriate for subsequent use. Store frozen, stable for 1 year. This volume can be scaled up or down as appropriate.

6. Prepare working calibrators to prepare 25 mL of each using volumetric glassware. Add approximately 10 mL certified negative plasma/serum to a labeled volumetric flask. Add the appropriate volume as shown in Table 1 of intermediate solution described in **item 5** above to the flask; q.s. to 25 mL using certified negative serum/plasma. Add a stir bar and stopper and mix for at least 30 min at room temperature. Aliquot as appropriate for future use. Store aliquots frozen, stable for 1 year. This volume can be scaled up or down as appropriate (*see* **Note 1**).

2.4 Controls and Internal Standard

1. Controls: May be purchased from a third party and prepared according to the manufacturer. They can also prepared in-house independently from calibrators' source material using Table 1 as a guideline (*see* **Note 1**).
2. Internal Standard (protein precipitation solution): Imipramine-D_3 100 mcg/mL in methanol (Cerilliant, Round Rock, TX). Add 250 mL of methanol to a 500 mL volumetric flask. Add 60 μL of reference material to the flask, QS to 500 mL with acetonitrile. Add a stir bar and a stopper. Mix for at least 30 min at room temperature. Aliquot as needed for use in this assay (volumes can be scaled up or down as appropriate). Store frozen, stable for 1 year (*see* **Notes 1** and **2**).

2.5 Supplies and Equipment

1. Instrument-compatible autosampler vials with injector appropriate caps.
2. Acquity HSS T3 1.8 μm, 2.1 × 50 mm UPLC column (Waters, Milford, MA).

3. Multi-tube Vortex mixer (e.g., VWR VX-2500).
4. Foam rack(s) compatible with both microcentrifuge tubes and multi-tube vortex mixer.
5. Centrifuge capable of 18,000 × *g* that will accommodate microcentrifuge tubes.
6. Waters Acquity TQD UPLC-MS/MS system (Milford, MA).

3 Methods

3.1 Stepwise Procedure

1. Briefly vortex or invert each sample to mix.
2. Aliquot 100 µL of each patient sample, calibrator and QC into appropriately labeled microcentrifuge tubes.
3. Add 300 µL of Internal Standard/precipitation solution to each vial.
4. Cap each tube and vortex vigorously for 30 s.
5. Centrifuge for ~10 min at ~18,000 × *g* (*see* **Note 3**).
6. Transfer the contents of each tube (from **steps 2** to **5**) to an autosampler vial and cap.
7. Analyze on LC-MS/MS.

3.2 Instrument Operating Conditions

1. Table 2 summarizes typical LC conditions.
2. Table 3 summarizes typical MS conditions.
3. Table 4 summarizes typical MRM conditions.

Each instrument should be individually optimized for best method performance.

3.3 Data Analysis

1. Representative MRM chromatograms of each antipsychotic and internal standard in plasma are shown in Fig. 1a–e.
2. The dynamic range for this assay is 0.2–12 ng/mL for fluphenazine and perphenazine and 1–60 ng/mL for haloperidol and thiothixene. Samples exceeding this range can be diluted 5× or 10× as needed to achieve an accurate calculated concentration, if needed.
3. Data analysis is performed using the QuanLynx or TargetLynx software to integrate peaks, calculate peak area ratios, and construct calibration curves using a linear $1/x$ weighted fit ignoring the origin as a data point. Sample concentrations are then calculated using the derived calibration curves (*see* **Note 1**).

Table 2
Typical HPLC conditions

Weak wash	Mobile Phase A				
Strong wash	Mobile Phase B				
Seal wash	Mobile Phase A				
Injection volume	8 µL				
Vacuum degassing	On				
Temperature	30 °C				
A Reservoir	0.1 % HCOOH in CLRW				
B Reservoir	0.1 % HCOOH in Acetonitrile				
Gradient table					
Step	Time (min)	Flow (µL/min)	A (%)	B (%)	Curve[a]
0	0	650	70	30	1
1	1	650	55	45	6
2	1.33	650	10	90	6
3	1.55	650	70	30	11

[a]Nonlinear gradient curves common to Waters systems

Table 3
Typical mass spectrometer conditions

Parameter	Value
Capillary (kV)	0.6
Cone (V)	42
Extractor (V)	3
RF (V)	0.3
Desolvation temp	450
Desolvation gas	900
Cone gas	30
Collision gas	0.25
Scan mode	MSMS
Polarity	Positive
Ion source	ESI
Resolution Q1	Unit
Resolution Q3	Unit
Dwell (s)	0.045

Table 4
Typical MRM conditions

Analyte	Precursor	Product (quant.)	Product (qual.)
Haloperidol	376.2 Collision energy	165.1 22	122.9 42
Fluphenazine	438.3 Collision energy	171.1 26	143.1 32
Perphenazine	404.2 Collision energy	143.1 28	171.11 24
Thiothixene	444.3 Collision energy	139.2 34	97.9 34
Imipramine-d_3 (internal standard)	284.2 Collision energy	89.1 16	193.1 42

4. Calibration curves should have an r^2 value $\geq$0.99.
5. Typical imprecision is <15 % both inter- and intra-assay.
6. An analytical batch is considered acceptable if chromatography is acceptable and QC samples calculate to within 20 % if their target values and ion ratios are within 20 % of the calibration curve ion ratios.

4 Notes

1. Validate/verify all calibrators, QCs, internal standard, and negative matrix pools before placing into use.
2. Imipramine-d_3 shows good recovery and a retention time intermediate to the other analytes making it a good compromise internal standard for all four antipsychotics. Ion suppression studies (data not shown) indicate that this I.S. offers good control of matrix effects under the conditions described. Deuterated analogs of each drug may be utilized if desired.
3. Time and speed of centrifugation step can be optimized to ensure a firm pellet is formed so as not to transfer any precipitate to autosampler vial.

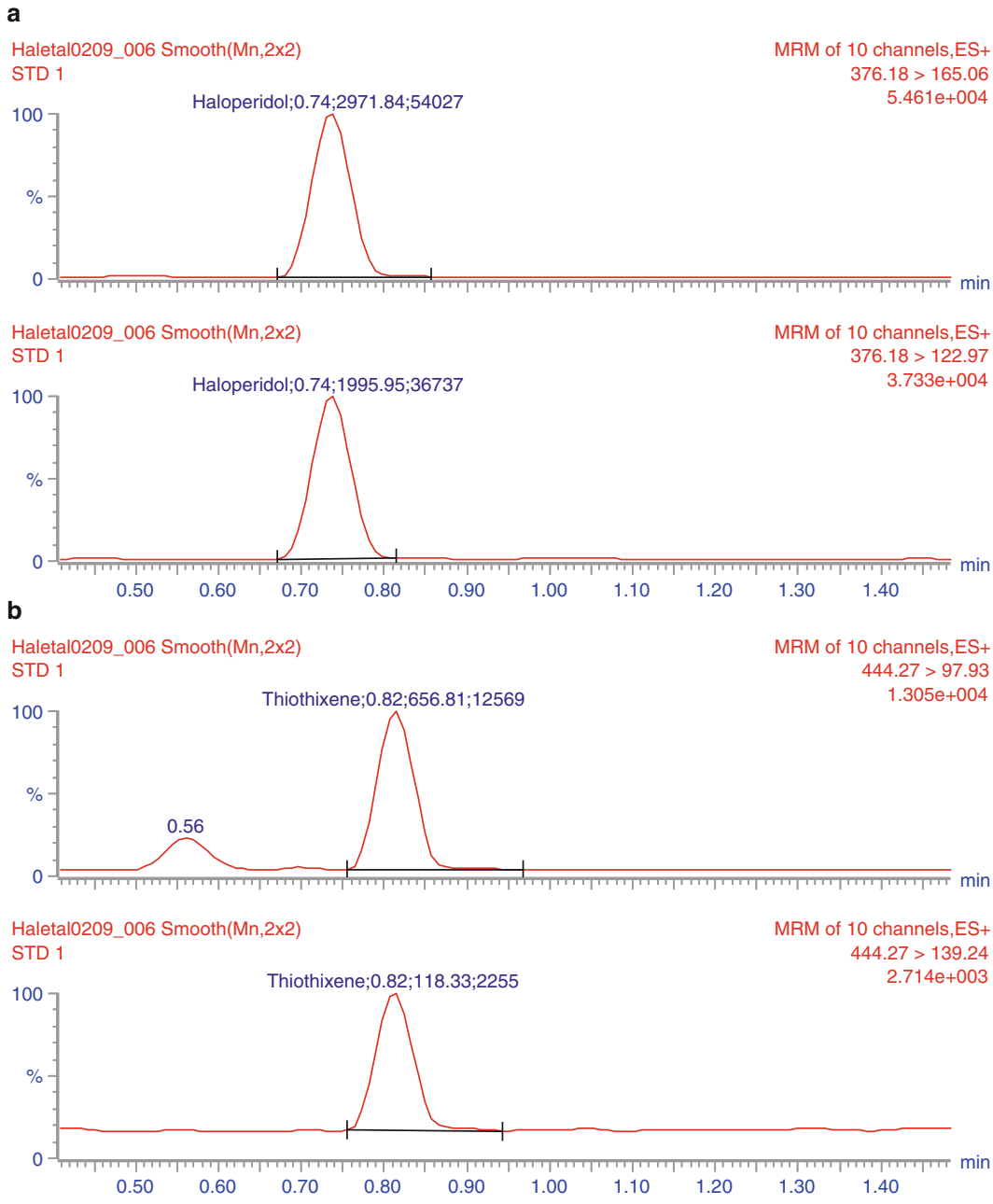

Fig. 1 Typical MRM chromatograms for (**a**) haloperidol (0.74 min), (**b**) thiothixene (0.82 min), (**c**) perphenazine (0.84 min), (**d**) imipramine-d3 (0.88 min), (**e**) fluphenazine (1.01 min) in plasma. 0.2/1 ng/mL (see text) extracted from fortified human plasma and analyzed according to the described method

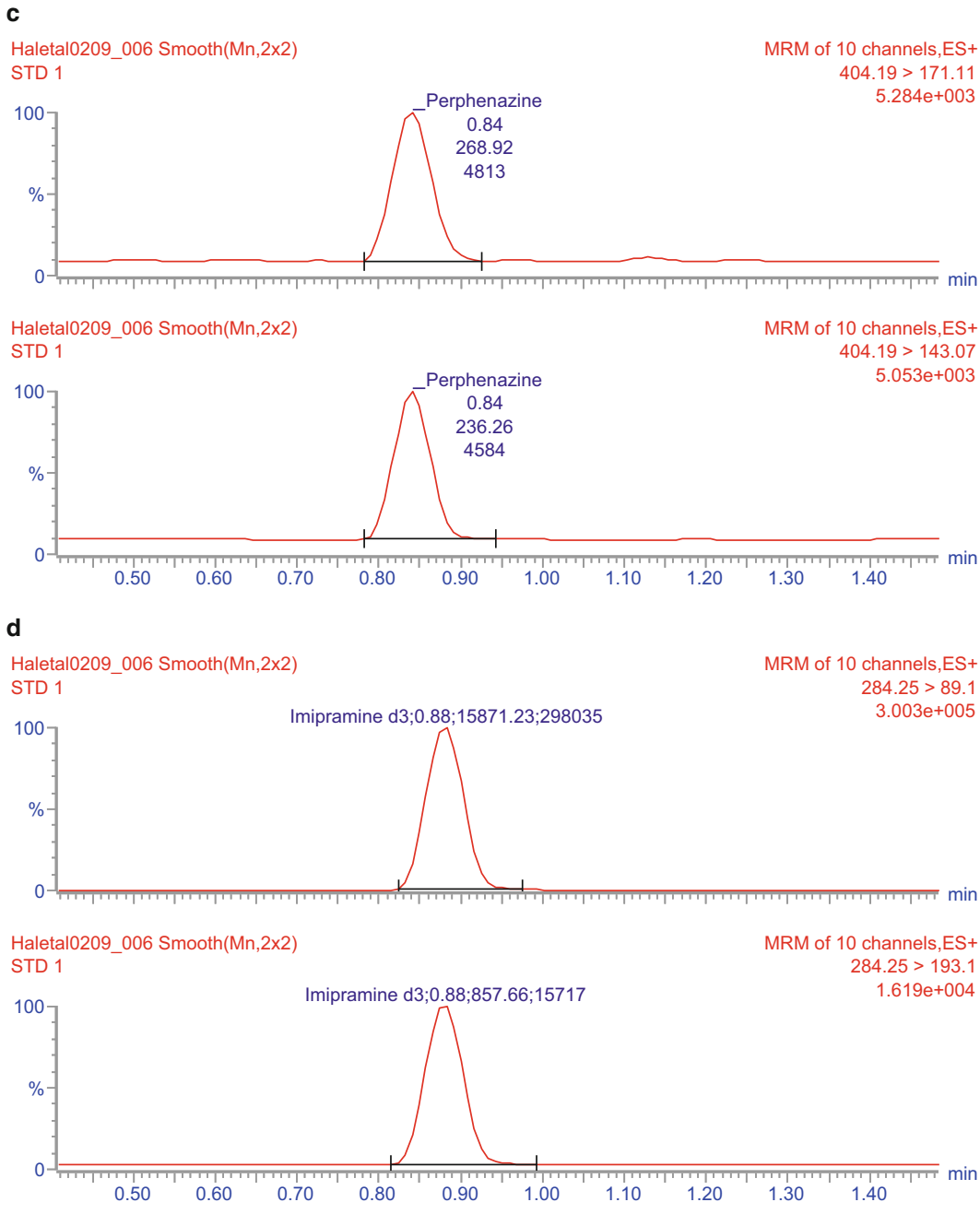

Fig. 1 (continued)

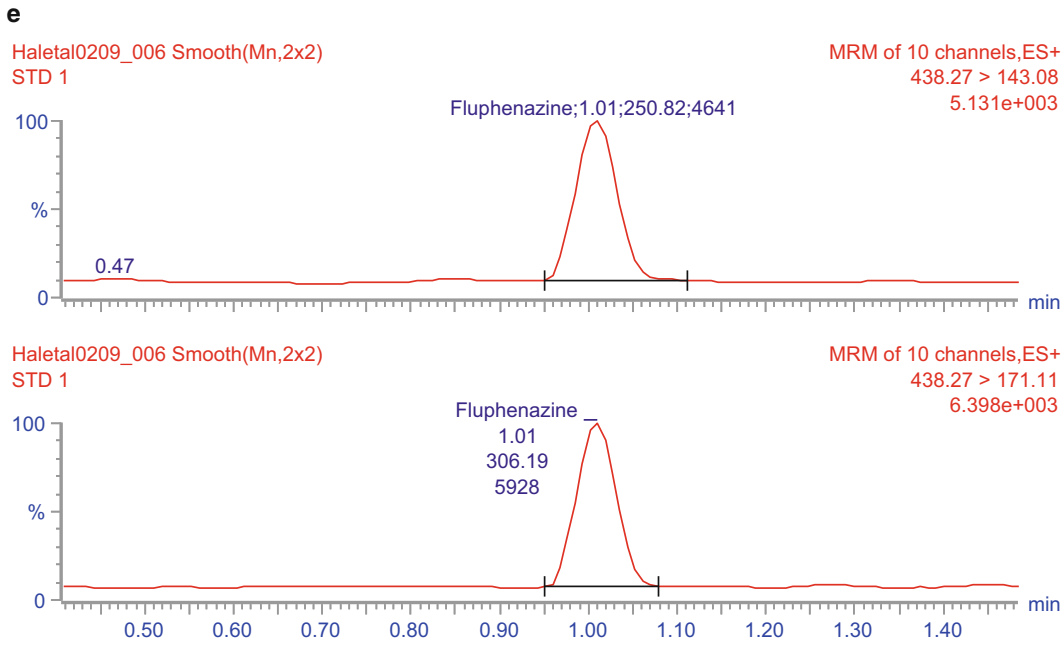

Fig. 1 (continued)

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