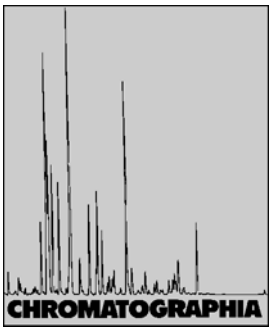
A Validated, Stability-Indicating Method for the Assay of Dexamethasone in Drug Substance and Drug Product Analyses, and the Assay of Preservatives in Drug Product



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Key Words

Column liquid chromatography
Dexamethasone
Degradation Products
Preservatives

Summary

A new high-performance liquid chromatographic (HPLC) procedure for the determination of dexamethasone, impurities, degradation products and product preservatives is described. A three-stage, linear gradient with UV detection at 240 nm allows the analysis of dexamethasone drug substance and dexamethasone in two formulated products, using the same chromatographic system. The Limit of Quantitation (LOQ) of dexamethasone impurities in drug substance is 0.05%, and 0.1% for dexamethasone degradation products in formulated products. The method is linear, precise, accurate and robust. Sample preparations are simple, and are accomplished without the use of an internal standard. Several degradation products of stressed dexamethasone have been identified.

Introduction

Dexamethasone is a potent fluorinated corticosteroid actively used as an anti-inflammatory agent, an immunosuppressive, to treat allergies and adrenal cortex insufficiency, induce parturition and alleviate stress.

An updated methodology to assay dexamethasone in dexamethasone drug substance and two finished products (a powder and an intravenous solution (IV)) was required to meet current analytical needs. The IV solution is an injectable dexamethasone solution also containing benzyl alcohol, methyl paraben and propyl paraben as preservatives.

Methods for directly assaying formulated dexamethasone-containing products are available in compendia and in the literature [1–13]. Some are more specific than others, many do not posses the sensitivity needed to satisfy current regulatory requirements. None of these methods could meet the current regulatory requirements and our need for a single, flexible system.

During our method development, top priority was given to complete separation of the impurities that were present in available drug substance lots. The 17-ketone of dexamethasone (a primary degradation product), along with the synthetic precursors/intermediates that were available (Figure 1), completed a library of compounds used to investigate system specificity. Consideration was given to developing a robust system suitable for the assay of dexamethasone-containing dosage forms and a system possessing adequate sensitivity to meet demanding limits of quantitation.

Experimental

Chemicals and Reagents

HPLC grade acetonitrile, 85% phosphoric acid, and glacial acetic acid were obtained from Fisher Scientific (Springfield, NJ, USA). Distilled, deionized water was prepared by a Milli-Q system (Millipore, Bedford, USA).

Liquid Chromatography Instrumentation

Both Hewlett-Packard (now Agilent) series 1100 and Waters Alliance liquid chromatographs were used for this work. HP 1100 systems included G1322A degassers, G1329A autosamplers, G1311A QuatPumps, G1316A column heater/chillers and G1314A variable wavelength and G1315A diode array ultraviolet detectors. ChemStation software revision A.07.01 controlled the systems. Waters 2690 Alli-

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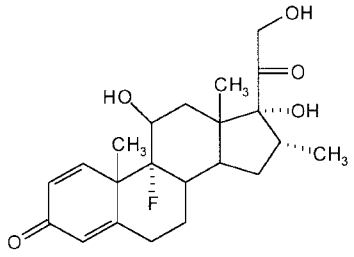
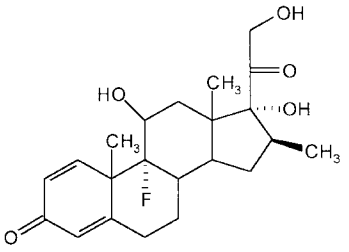
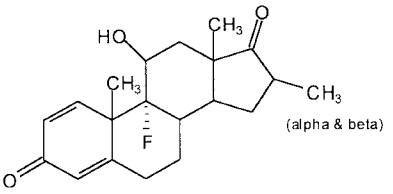


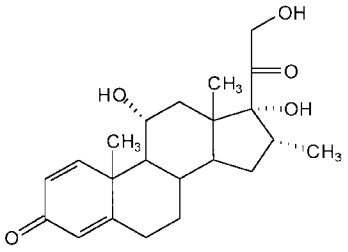
A. Dexamethasone



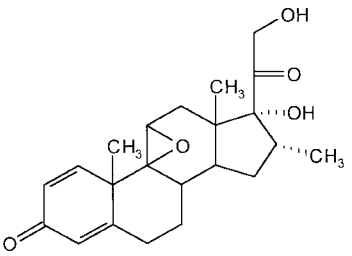
B. Betamethasone
(epimer of dexamethasone)



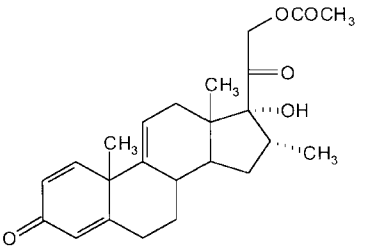
C. 17-Ketone
(degradation product
of dexamethasone)



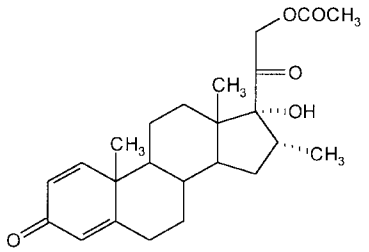
D. DOB-AA



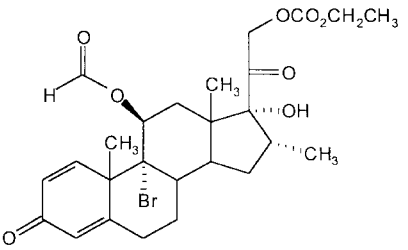
E. DOB-MN



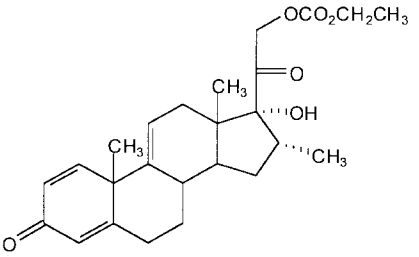
F. Pregnatriene acetate



G. DOA-R



H. DOB-JN



I. DOB-F

Figure 1. Structures of dexamethasone-related compounds.

ance systems were fitted with a Waters 2487 dual wavelength ultraviolet detectors and a Jones (Lakewood, CO, USA), model 7955 column heater/chiller. Millennium 32 software revision 3.05.01 controlled the Waters system. Chromatographic data was acquired and processed by Turbochrom 4.1.

Chromatographic System

The chromatographic system utilized a binary gradient. Mobile phase A consisted of acetonitrile:water:phosphoric acid (100:900:0.5, *v/v/v*). Mobile phase B contained acetonitrile:water:phosphoric acid (900:100:0.5, *v/v/v*). See Table I for the gradient program to achieve gradient elution using mobile phases A and B described above.

A YMC (°/o Waters, Milford, MA, USA) J'sphere, ODS-H80, 250 × 4.6 mm ID, 4 µm, 80 Å analytical column, held isothermally at 25 ± 2 °C, was used to effect the separation. Injection volumes equaled 25 µL. Detection was at 240 nm and total run time ran to 85 minutes.

Preparation of Standard and Samples

Nominal standard and sample concentrations for HPLC injection were targeted to 120 µg mL^{-1}. Dexamethasone reference standard was dissolved in acetonitrile, and then diluted (allowing for temperature equilibration of mixed solvents) with acidic water to produce 20% acetonitrile, 0.05% phosphoric acid – mobile phase composition at the time of injection. Dexamethasone drug substances samples were similarly prepared.

Powder samples were vigorously vortexed with acetonitrile, centrifuged and filtered. The filtrate was similarly diluted with acidic water before analysis. Standards for IV solution sample analysis were prepared from stock standard solutions of benzyl alcohol, methyl paraben, propyl paraben and dexamethasone dissolved in acetonitrile, and diluted with acidic water as described previously. IV solution samples were directly diluted with 20% acetonitrile, 0.05% phosphoric acid.

Table II shows the stress conditions and solvents used to prepare samples of stressed dexamethasone drug substance, powder, and IV solution samples for assay. Controls, placebo preparations and blanks were also prepared and assayed. Cohen projected possible degradation

pathways in his profile of dexamethasone in Analytical Profiles [14]. Several major photodegradation products of dexamethasone were reported by Fahmy [15]. Several acid, base, light and oxidation degradation products of stressed betamethasone (the epimer of dexamethasone) were reported by Hidaka et.al. [16].

Mass Spectroscopy Instrumentation

The mobile phase A consisted of acetonitrile:water:acetic acid (100:900:0.5, *v/v/v*). Mobile phase B contained acetonitrile:-water:acetic acid (900:100:0.5, *v/v/v*). The LC/MS system used employed the same gradient program as the assay systems, however a flow rate of 1.4 mL min^{-1} was maintained throughout the program (Table I). Mass spectra were generated from a HP 1100 system coupled with a G1946B LC/MSD operated in the positive mode. Ionization was by API-ES, masses were scanned from 50 to 700 AMUs, gas temperature was 350 °C at 13.0 L min^{-1}, using a nebulizing pressure of 60 psi and a voltage of 3000 V. Data in the condensed mode, was acquired by the ChemStation controller.

Table I. Gradient program.

Time (min)	Flow rate (mL min^{-1})	Mobile phase A	Mobile phase B
0	1.4	87.5%	12.5%
5.0	1.4	81.3%	18.7%
35.0	1.4	75.0%	25.0%
70.0	1.4	30.0%	70.0%
70.1	2.0	87.5%	12.5%
83.0	2.0	87.5%	12.5%
83.1	1.4	87.5%	12.5%

Mobile phase A: 900:100:0.5, (*v/v/v*), water:acetonitrile: phosphoric acid. Mobile phase B: 100:900:0.5, (*v/v/v*), water:acetonitrile: phosphoric acid.

Table II. Stress conditions for dexamethasone drug substance, powder samples and IV solution samples.

Solvent	Stress conditions
Dexamethasone drug substance	
20% ACN, 80% 0.1 N HCl	0.1 N HCl, 75 °C, 20 hours
20% ACN, 80% 0.01 N NaOH	0.01 N NaOH, 2 hours
20% ACN, 80% 3.75% H_2O_2	3% H_2O_2, room temperature, 6 days
20% ACN, 80% H_2O	UV light, 366 nm, 70 minutes
None	Melt (solid), 255 °C, 8 minutes
20% ACN, 80% H_2O	Heat (solution), 75 °C, 1 week
None	Aged drug substance
Powder	
None	Heated powder, 75 °C, 1 week
20% ACN, 80% H_2O	ICH photoexposure
None	Aged powder
IV Solution	
None	Heated Solution, 75 °C, 10 days
None	UV light, 366 nm, 60 minutes
None	Aged solution

Method Validation

The assays of dexamethasone drug substance, powder and the IV solution were validated according to internationally accepted criteria.

System specificity was confirmed by assuring resolution ($R_s \geq 2$) of critical dexamethasone-related compounds from each other, from the preservatives and of the observable stress-induced degradation peaks. Specificity was also supported by the diode array confirmation of the dexamethasone peak purity of stressed and aged samples of dexamethasone drug substance, powder and IV solution. System robustness was examined by varying one chromatographic parameter at a time while monitoring critical system suitability parameters. Variations included different lots of column packing material, column temperature, injection volume, flow rate, detection wavelength, mobile phase acid concentration, mobile phase organic content, delays in gradient start time, and more gradual or steeper gradient slopes.

Dexamethasone linearity was demonstrated at 50% to 150% assay concentration; dexamethasone and its precursor, DOB-MN linearity were demonstrated from LOQ to 5% of the nominal assay concentration. Linearity of the preservatives at assay level was confirmed as well. System precision was determined concurrently on three different systems; method precision of assay preparations was determined by two different analysts. Assay levels (50% to 150%) of dexamethasone were recovered from spiked powder placebo, and dexamethasone and preservatives recovered from spiked IV solution placebo. LOQ to 2.5% levels of DOB-MN were recovered from dexamethasone drug substance spiked powder and IV solution placebos containing dexamethasone, all at nominal (product) potency. Solution stability and comparability studies (results from the new assay methodologies versus results from the existing procedures) were also included in the validation of the proposed assay method.

Results and Discussion

Typical chromatograms for the assay of dexamethasone drug substance, powder and IV solution are presented in Figure 2. A mixture of all available dexamethasone relateds and IV solution preservatives is shown in Figure 3. This preparation, along with sample preparations, was injected under all of the robustness conditions.

The chromatographic system was found to be robust, maintaining its specificity, efficiency and resolving power throughout all but one of the robustness conditions. A lowered column temperature (20 °C), produced a notable decrease in separation efficiency, distinguished by a drop in the apparent theoretical plate values for eluting peaks. The system suitability criteria of resolution (Rs $\geq$ 2.0) between the epimers dexamethasone and betamethasone, would detect and reject any system that did not have adequate temperature control. Diode array analysis confirmed the homogeneity of the dexamethasone peak in stressed and aged drug

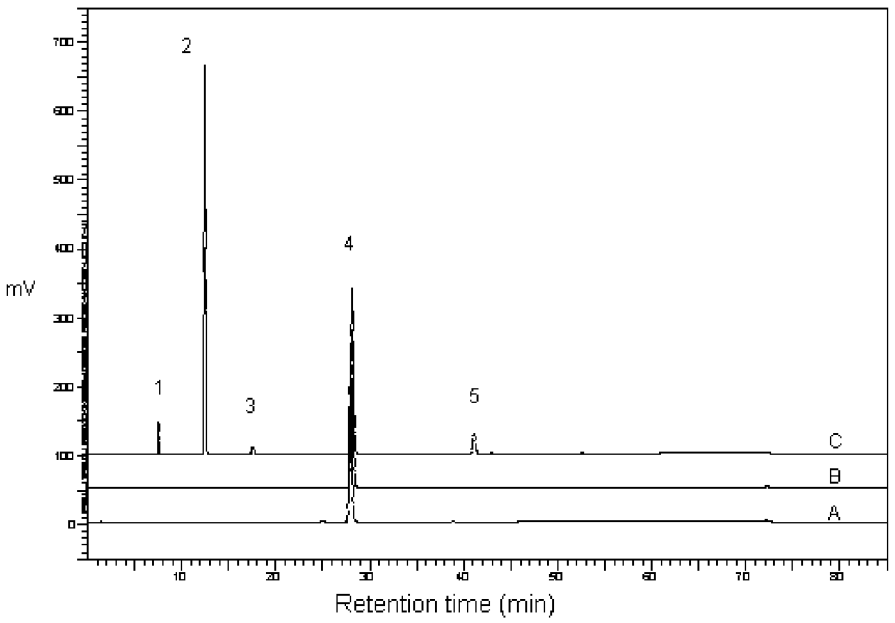


Figure 2. HPLC chromatogram of dexamethasone drug substance, powder and IV solution assays. Peak identification: **1** = Benzyl alcohol, preservative; **2** = Methyl paraben, preservative; **3** = Benzaldehyde, degradation product of benzyl alcohol; **4** = Dexamethasone, active; **5** = Propyl paraben, preservative. Chromatogram: **A**, Dexamethasone; **B**, Powder; **C**, IV Solution.

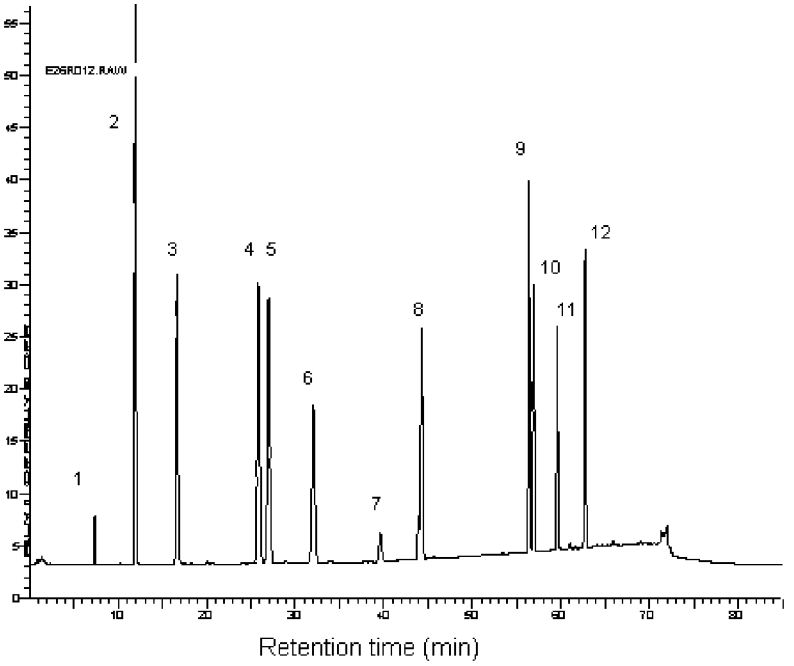


Figure 3. HPLC chromatogram of the robustness solution. Peak identification: **1** = Benzyl alcohol; **2** = Methyl paraben; **3** = DOB-AA; **4** = Betamethasone; **5** = Dexamethasone; **6** = DOB-MN; **7** = Propyl paraben; **8** = 17-Ketone; **9** = Pregnatriene acetate; **10** = DOA-R; **11** = DOB-JN; **12** = DOB-F.

substance and formulated product samples.

The assays of dexamethasone drug substance, powder and IV solution were examined, and dexamethasone and the preservatives were found to be linear at 50–150% the nominal assay concentrations, both with and without the presence of placebos (Table III). Measures of system and method precision were below 0.4% and 0.5% RSD respectively at assay concentrations and below 6.5% and 8.9% RSD respectively at LOQ levels, for all of the validation data generated for the three analytical methods being examined.

The stability of assay solutions for a minimum of three days was established. Low level standards and LOQ solutions are always to be prepared fresh daily. Method comparability of assay and the determination of degradation products data was also confirmed. The proposed methods were generally more specific, separating a greater number of impurities/degradation products.

The recovery of dexamethasone, each of the preservatives and DOB-MN was confirmed for each assay, never falling below an average of 98.9% recovered at assay level, or below 81.7% recovered for an impurity at the lowest LOQ level. The required limits of quantitation (0.05% for drug substance assay, 0.1% for drug product assays) were achieved. Based on analyst-to-analyst assay comparability, no sample preparation issues are expected to arise during future method transfers. Substandard equipment however, may not exactly reproduce the three-stage linear gradient required. The ruggedness of the method to withstand a delay in the start of the gradient program should maintain the integrity of the separation, despite a chromatographic system with excess dwell volume.

Based on molecular weights and fragment masses obtained from single quadrapole LC-MS analysis and relevant literature references, tentative structures were proposed for several of the observed stress-induced degradation products of dexamethasone (Figure 4 and Table IV). Electrospray and in-source collision induced dissociation (CID) fragment ion data (loss of HF, CO and H_2O groups) supported the proposed structures. Two of the structures were additionally confirmed against the retention times and spectra of standards.

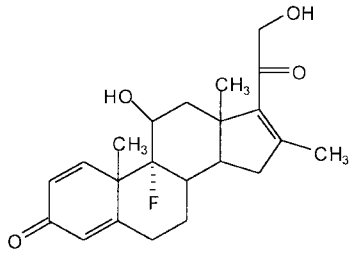
Conclusion

The reverse phase, three-stage linear gradient developed by Schering-Plough was proved to be suitable for the assay of dexamethasone and chromatographic impurities in dexamethasone drug substance. It was also shown to be flexible enough to assay dexamethasone, degradation products and preservatives in powder and IV solution dosage forms. The identification of several, stress-induced dexamethasone degradation products furthered the understanding of dexamethasone drug substance and formulated product stability.

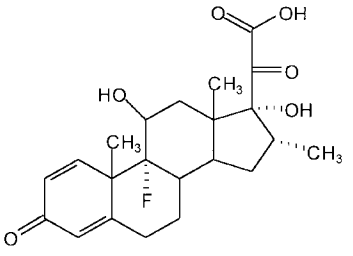
Table III. Linearity of assay procedures.

Sample	Validation	Compound	Range ($\mu g\,mL^{-1}$)	n	Equation[1)]	Standard error[2)]	$R^{2)}$
Drug substance & powder	Linearity	Dexamethasone	60–180	5	$y = 40{,}157\,x + 41{,}987$	14,104	0.99996
Drug substance & powder	Linearity	Dexamethasone	0.05–6	5	$y = 40{,}579\,x - 157$	319	0.99999
Drug substance & powder	Linearity	DOB-MN	0.05–6	5	$y = 32{,}730\,x - 41$	386	0.99998
Powder	Recovery from placebo	Dexamethasone	60–180	9	$y = 40{,}078\,x + 36{,}719$	11,159	0.99997
Powder	Recovery from placebo	DOB-MN	0.06–2.4	12	$y = 32{,}707\,x + 213$	565	0.99972
IV Solution	Linearity	Dexamethasone	60–180	5	$y = 40{,}374\,x - 8{,}861$	6,707	0.99999
IV Solution	Linearity	Benzyl alcohol	270–810	5	$y = 554\,x + 2244$	799	1.00000
IV Solution	Linearity	Methyl paraben	54–162	5	$y = 52{,}823\,x + 79{,}280$	17,494	0.99996
IV Solution	Linearity	Propyl paraben	6–18	5	$y = 46{,}417\,x - 324$	825	0.99999
IV Solution	Linearity	Dexamethasone	0.05–3	5	$y = 40{,}708\,x + 178$	100	1.00000
IV Solution	Linearity	DOB-MN	0.05–3	5	$y = 33{,}214\,x + 182$	307	0.99996
IV Solution	Recovery from placebo	Dexamethasone	60–180	9	$y = 40{,}307\,x + 186$	9,964	0.99998
IV Solution	Recovery from placebo	Benzyl alcohol	270–810	9	$y = 554\,x + 2{,}884$	487	0.99999
IV Solution	Recovery from placebo	Methyl paraben	54–162	9	$y = 52{,}743\,x + 92{,}225$	19,495	0.99995
IV Solution	Recovery from placebo	Propyl paraben	6–18	9	$y = 46{,}477\,x + 459$	1,062	0.99998
IV Solution	Recovery from placebo	DOB-MN	0.05–3	12	$y = 33{,}415\,x + 50$	177	0.99998

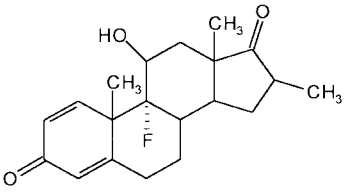
[1)] Regression line (y = mx + b); y = peak area, x = concentration, $\mu g\,mL^{-1}$. [2)] Standard error = estimated error of fit (area units).



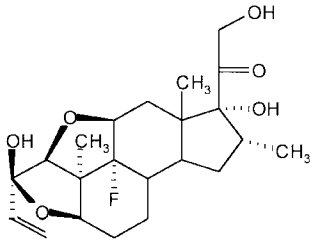
Structure 2, MW = 374



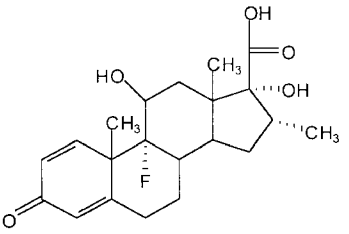
Structure 3, MW = 406



Structure 4, MW = 332
17- Ketone (confirmed against a standard)



Structure 5, MW = 410



Structure 7, MW = 378
(confirmed against a standard)

Figure 4. Proposed structures of stress-induced degradation products. (Structure numbers as in Table IV).

The proposed procedure(s) fulfills the current regulatory/analytical requirements for the analysis of dexamethasone and two dexamethasone-containing formulated products with a single, harmonized methodology.

Acknowledgments

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Table IV. Stressed Dexamethasone drug substance, powder and IV solution.

Stress condition	Unknown number	RRT[1)]	Molecular weight		Structure number
Acid	1	1.27	392		1
Acid	2	1.82	374		2
Oxidation	1	1.47	406		3
Oxidation	2	1.56	332	17-Ketone	4
UV	1	0.35	410		5
UV	2	0.57	406		
UV	3	0.66	424		
UV	4	0.90	392	Not betamethasone	
UV	5	1.38	392		
UV	6	1.56	332	17-Ketone	4
UV	7	1.66	392		
Melt	1	0.54	392		
Melt	2	1.39	360		
Melt	3	1.41	374		
Melt	4a	1.56	332	17-Ketone	4
Melt	4b	1.57	332	17-Ketone	4
Melt	5	1.82	374		2
Heat	1	0.89	408		6
Heat	2	1.27	392		1
Heat	3	1.47	406		3
Heat	4	1.55	332	17-Ketone	4
Heat	5	1.59	362		
Heat	6	1.81	374		2
Base	1	0.72	433		
Base	2	0.88	408		6
Base	3	1.05	408		
Base	4	1.16	378		7
Base	5	1.48	406		3
Base	6a	1.56	332	17-Ketone	4
Base	6b	1.57	332	17-Ketone	4

[1)] Retention times relative to dexamethasone are reported from the chromatographic system described in *Mass Spectroscopy Instrumentation.*

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