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Journal of Chromatography B, 707 (1998) 181–187

JOURNAL OF CHROMATOGRAPHY B

Determination of methylparaben, propylparaben and chlorpromazine in chlorpromazine hydrochloride oral solution by high-performance liquid chromatography

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Received 11 June 1997; received in revised form 2 December 1997; accepted 2 December 1997

Abstract

A reversed-phase high-performance liquid chromatographic assay method was developed and validated for the simultaneous determination of methylparaben and propylparaben preservatives and chlorpromazine hydrochloride active component in a liquid oral pharmaceutical formulation. The method separated the analytes as well as some degradants and other components, providing good resolution and moderate tailing. The performance of various C_{18} columns was compared. There were significant differences in selectivity and only a few phases showed acceptable tailing without the addition of triethylamine modifier in the mobile phase. © 1998 Elsevier Science B.V.

Keywords: Methylparaben; Propylparaben; Chlorpromazine

1. Introduction

Chlorpromazine (Fig. 1) in its hydrochloride form is widely used in the treatment of psychotic disorders.

The current United States Pharmacopeia (USP) methods of assay for chlorpromazine in various formulations are utilizing spectrophotometry or titration, while the purity is checked by thin-layer chromatography [1].

Recently there have been a number of reports dealing with the determination of this compound by liquid chromatography using octadecyl [2], octyl [3,4] and C≡N phases [5].

Chlorpromazine, a basic compound, typically shows poor chromatographic behavior resulting in

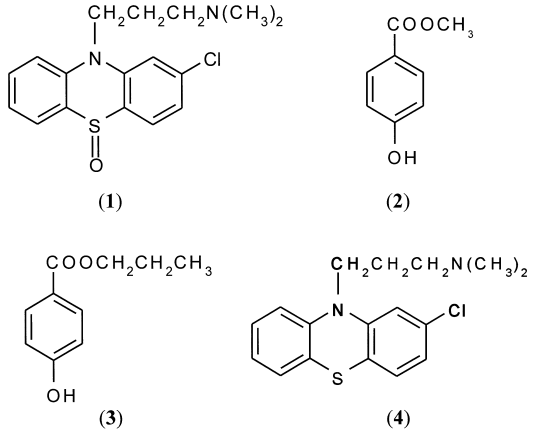

Fig. 1. Structures of the analytes, in order of elution: 1 - chlorpromazine sulfoxide; 2 - methylparaben; 3 - propylparaben; 4 - chlorpromazine.

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0378-4347/98/$19.00
PII S0378-4347(97)00618-X

broad, tailing peaks when analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC). Various mobile phase modifiers, such as short-chained amines and ion-pair reagents have been used to obtain acceptable chromatograms with compounds of this type [6].

In our laboratory, we have been using separate HPLC methods for the assay of the active component and preservatives in chlorpromazine hydrochloride oral solution. Ion-pair reagents were added to the mobile phase in the former case to improve the peak shape of chlorpromazine [7], which also increased the retention time of chlorpromazine substantially.

Due to the nearly hundredfold difference between the chlorpromazine and paraben concentration in the product during the assay of the parabens in a moderately diluted sample (dilution factor 10–20), the chlorpromazine seriously overloads the column. Under these conditions, the typical 10–30 m*M* PIC-reagent concentration is not sufficient to reduce the tailing to an acceptable level. As a result, the analysis time increases excessively since the analyst has to wait until the system clears out.

Some silanol-deactivated and high-purity silica based C_{18} columns – according to the manufacturer's claims – eliminate the problems of both acid and base related tailing without additives [8–10]. Although these columns are now available from numerous sources, they have not yet been incorporated in many official methods. One reason for this may be the end-users' worry about the useful lifetime of these phases.

The goal of the present study was to develop a single method for the simultaneous determination of methylparaben, propylparaben and chlorpromazine with improved overall chromatography. We examined the performance of various specialty columns and compared them to the regular C_{18} phases from the same manufacturer to select the one most suitable for this particular application.

2. Experimental

2.1. Materials

The in-house secondary standards used during this study have been assayed several times against USP reference standards: methylparaben, propylparaben, chlorpromazine hydrochloride. Chlorpromazine sulfoxide was prepared by a USP procedure [1].

The organic solvents were HPLC grade (Burdick and Jackson, Muskegon, MI, USA). The deionized and carbon-filtered water was purified in-house to meet USP specifications. All other chemicals were reagent grade from Mallinckrodt, (Paris, KY, USA) and were used without further purification.

The mobile phase was prepared by dissolving 3.402 g sodium acetate trihydrate and 2 ml triethylamine in 970 ml purified water. The pH of the solution was adjusted to 4.5 with glacial acetic acid and diluted to 1000 ml with purified water. A 600-ml volume of this solution was mixed with 180 ml HPLC grade methanol and 220 ml HPLC grade acetonitrile.

Standard solutions were prepared in mobile phase containing 0.075 mg ml^{-1} methylparaben, 0.01 mg ml^{-1} propylparaben, 0.05 mg ml^{-1} chlorpromazine and 0.0025 mg ml^{-1} chlorpromazine sulfoxide.

A lab-scale placebo was prepared in 1 l size. It contained neither chlorpromazine nor preservatives. A lab-scale finished product was also prepared containing 100 mg ml^{-1} chlorpromazine hydrochloride, 1.5 mg ml^{-1} methylparaben and 0.2 mg ml^{-1} propylparaben in a sugar-based syrup. The finished product samples were serially diluted in mobile phase to achieve dilution factors 20 and 2000. The parabens were quantitated using results from the first dilution, while chlorpromazine and chlorpromazine sulfoxide were quantitated using the results from the most diluted solutions.

Low actinic glassware was used for all sample and standard preparations.

2.2. Instrumentation and columns

A Hewlett–Packard (Palo Alto, CA, USA) 1050 HPLC system, consisting of a quaternary pump, a de-gasser, a column heater, an autoinjector and a diode-array detector was controlled by a Vectra 486/66XM computer and HP-Chemstation software (DOS, Rev. A.02.02).

A Shimadzu (Kyoto, Japan) HPLC system consisting of a SIL-10A autoinjector, a LC-10AS pump, a

SPD-10 AV UV detector and a CR 501 integrator was used in the ruggedness test.

The 15 cm×4.6 mm (in the case of Waters columns 3.9 mm), 5 μm particle size columns used during this study are listed in Table 1. With the exception of Phenomenex, a regular C_{18} and a silanol-deactivated or 'high purity' special C_{18} phase was tested from each supplier. While several columns appeared to be suitable for the analysis, the Waters 15 cm×3.9 mm Symmetry C18 column was selected as final choice for our HPLC method.

2.3. Analytical operating parameters

The finally selected conditions were as follows: flow-rate 1.0 ml min^{-1}, detection wavelength 254 nm, injection size 20 μl, column temperature 25°C. Fig. 2 shows chromatograms of a standard and a sample solution.

The chromatograms were interpreted by reference to retention times of pure standards. Peak identity was confirmed by comparison of the UV–visible spectra with an in-house computerized spectral library.

2.4. Method validation

The method was validated by a standard procedure to evaluate if adequate accuracy, precision, selectivity and linearity had been achieved. Accuracy was determined using spiked placebo solutions at three levels, three preparations each.

Relative standard deviation (R.S.D.) values were calculated for repeated sample and standard injections (system precision) as well as repeated injections of multiple sample preparations (method precision).

Linearity was determined in the 25% to 150% range with duplicate standard preparations at six levels.

Visual inspection of chromatograms of standard and placebo solutions was conducted to ensure specificity. Peak homogeneity was checked by spectral comparison using a diode-array detector on the peaks of interest. Short-term stability of standard and sample solutions was evaluated by comparison of response factors of fresh and stored standard and sample solutions.

Placebo, standard and sample solutions were also subjected to acidic, basic, oxidative, thermal and photolytic stress to monitor possible interference from degradation products. Chemical stress was performed at reagent concentrations of 0.1 *M* for HCl and KOH and 0.1% for hydrogen peroxide for 3 h. In the photolytic studies sample, standard and placebo solutions were exposed to regular laboratory illumination for 24 h. Thermal stress was performed at 70°C for 6 h.

Ruggedness or intermediate precision was determined by a second analyst, on a different day, using a different lot of the same type of column, different instrument, and the same lot of materials, performing

Table 1
Columns used during the study

Supplier	Location	Column	Lot or column ID
Waters[a]	Milford, MA, USA	Symmetry C_{18}	T41812
			T43251
		μ-Bondapak C_{18}	T42931
Supelco	Bellefonte, PA, USA	Supelcosil LC-ABZ	#250970AB
		Supelcosil LC-18	004455AE
		Supelcosil ABZ+PLUS	#323592AA
YMC	Wilmington, NC, USA	Basic B-02-5	DI01333
		ODS-A	ED18490
Alltech	Deerfield, IL, USA	Adsorbosphere UHS C18	#94061081
		Alltima C_{18}	#2259
Phenomenex	Torrance, CA, USA	Inertsil ODS-2	#85806
		Prodigy ODS-2	#94459

All columns were 4.6×150 mm, packed with 5 μm particle size octadecyl phases, unless otherwise noted.
[a] The Waters columns were 3.9×150 mm.

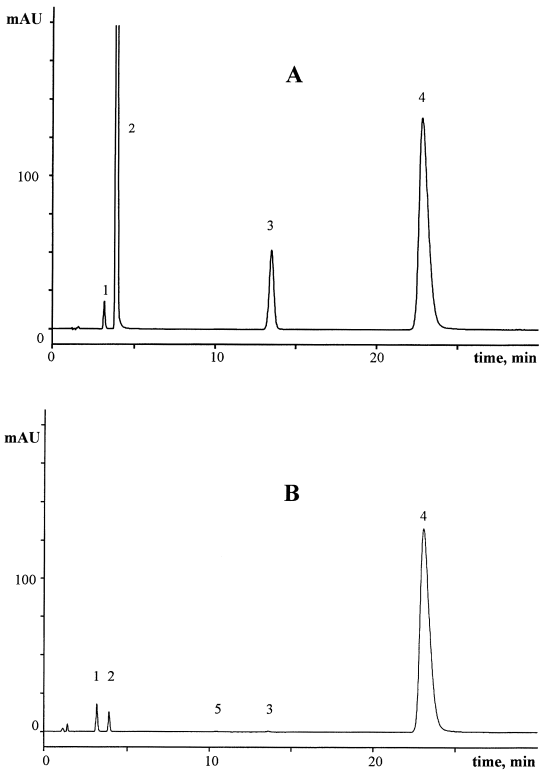

Fig. 2. (A) Chromatogram of a standard solution. Peaks and corresponding analyte concentrations: (1) chlorpromazine sulfoxide 2.5 $\mu g\,ml^{-1}$; (2) methylparaben 75 $\mu g\,ml^{-1}$; (3) propylparaben 10 $\mu g\,ml^{-1}$; (4) chlorpromazine hydrochloride 50 $\mu g\,ml^{-1}$. (B) Chromatogram of a sample solution, dilution×2000, spiked with chlorpromazine sulfoxide. Peaks and corresponding analyte concentrations: (1) chlorpromazine sulfoxide 2.5 $\mu g\,ml^{-1}$; (2) methylparaben 0.75 $\mu g\,ml^{-1}$; (3) propylparaben 0.1 $\mu g\,ml^{-1}$; (4) chlorpromazine hydrochloride 50 $\mu g\,ml^{-1}$; (5) impurity in chlorpromazine hydrochloride, less than 0.1 area percent. Chromatographic conditions: as in Table 2.

system precision, method precision and linearity tests. The results of the two analysts were compared to detect any significant difference.

3. Results and discussion

Kiel and his co-workers [3] carried out a systematic optimization of their method for the separation of chlorpromazine and many structurally related compounds (mostly metabolites). Unfortunately, their method was not directly applicable to our situation. During our study, we encountered only two potentially interfering compounds related to chlorpromazine, chlorpromazine sulfoxide (an oxidation product) and an unidentified trace impurity of the raw material. At the same time, the preservatives and *p*-hydroxy benzoic acid (a paraben degradation product) also had to be separated from the chromatographically quite different chlorpromazine.

The same was true for other recent publications [4,5]. There the greatest difficulties to the chromatographers were represented by the matrix (e.g. breastmilk and plasma) as well as the low detection limit targets. In contrast during the routine quality control of liquid formulations, detection limits typically do not represent a problem and matrix interferences are often limited to the color and flavor components of the sugar-based syrups and elixirs.

Our original chlorpromazine assay method required a Waters μ-Bondapak C_{18} column. Preliminary tests indicated coelution problems with both octyl and phenyl phases, so we decided to improve the method starting with the original conditions and limiting our investigation to C_{18} phases. Mobile phase composition and pH were modified and columns were changed until the chromatograms showed both adequate separation and acceptable tailing.

Table 2 lists the retention times of the three major components under the final conditions, as stated in the experimental part. Since the carbon load and other physical parameters of the phases are comparable, the differences in chromatography are the consequence of the differences in the silica matrix. Modifications to the phase-such as endcapping, sterical protection or electrostatic shielding of the silica-also affect the selectivity and tailing. None of the columns investigated had polymer coating on the silica.

The different columns, arranged in Table 2 by increasing order of chlorpromazine elution show a similar general trend for parabens as well. The separately listed Supelco columns are clearly different from the rest, since chlorpromazine is retained very strongly on the regular C_{18} phase, but the parabens are eluting earlier than on the ABZ column.

With Waters, the retention time of propylparaben decreases while methylparaben and chlorpromazine elute later when switching from Symmetry to the regular C_{18} column. In the case of Alltech, both

Table 2
Retention times (min) on various C_{18} phases

Supplier	Column Type	Methylparaben	Propylparaben	Chlorpromazine
Waters	Symmetry C_{18}	3.8	13.3	22.0
YMC	Basic B-02-5	4.6	11.5	22.6
Waters	Bondapak C_{18}	4.2	11.8	27.6
Phenomenex	Inertsil ODS-2	5.1	17.6	30.5
Phenomenex	Prodigy ODS-2	5.4	18.8	31.1
YMC	C_{18}	5.7	19.7	38.5
Alltech	Adsorb. UHS	6.9	27.4	49.3
Alltech	Alltima C_{18}	6.5	23.7	50.9
Supelco	LC-ABZ	5.7	17.2	14.3
Supelco	ABZ+PLUS	5.9	18.5	16.3
Supelco	C_{18}	4.6	14.9	70.0

Mobile phase: (3.4 g l^{-1} sodium acetate trihydrate and 0.2% v/v triethylamine in water, pH 4.5)–methanol–acetonitrile=60:18:22, 1.0 ml min^{-1}, 20 μl injection, UV detection at 254 nm.

parabens elute earlier from the specialty Alltima column but chlorpromazine elutes later than from Adsorbosphere.

The strong retention of chlorpromazine on the Supelco column is fairly typical for the type of silica used in manufacturing and can be explained with silanol interactions. A detailed analysis of the other two exceptions would involve details of the manufacturing process, about which the suppliers often reveal little, although some disclosed parameters, like the type and purity of the silica support undoubtedly have a major impact on selectivity.

It is difficult to make a meaningful overall comparison based on retention times only, since some of the phases were end-capped, while others were simply manufactured from high-purity components under more strictly controlled conditions than the regular C18 phases.

An indication of the effectiveness of the end-capping technique or the purification of the materials used in the column manufacturing process is the tailing factor of the chlorpromazine peak. Table 3 shows tailing factors obtained with some of the columns with reasonable retention times. Note that without triethylamine only two of the columns provide good peak shape.

Despite the manufacturers claims, all columns showed improvement when triethylamine was added to the mobile phase. Under the final conditions selected only one column failed to give the required tailing factor (<2). The tailing factors for the parabens were between 1.0 and 1.1 on all phases mentioned in this study.

Based on selectivity, peak shape, analysis time and batch-to-batch reproducibility the Symmetry column was selected as the final choice for this particular application. The importance of selectivity is apparent if we look at the Supelco ABZ column, where the propylparaben elutes after chlorpromazine. Accurate quantitation of a small peak riding on the tail of a bigger one is very difficult, as was demonstrated by computer simulation [11].

Another column suitable for the analysis is the recently introduced Prodigy, which showed the smallest tailing among all phases compared, although the uncorrected retention times are somewhat longer than on the Symmetry. (Note that the column diameters and consequently the linear flow-rates are not the same).

While it is general practice to compare various

Table 3
USP tailing factors for chlorpromazine with or without 0.2% v/v triethylamine (TEA) in the mobile phase

Supplier	Column Type	with TEA	no TEA
Phenomenex	Prodigy ODS-2	1.3	1.5
Supelco	LC-ABZ	1.6	1.9
Phenomenex	Inertsil ODS-2	1.5	2.1
YMC	Basic B-02-5	1.5	2.2
Waters	Symmetry C_{18}	1.5	2.7
Waters	Bondapak C_{18}	1.4	2.9
Alltech	Alltima C_{18}	2.0	2.9

Other conditions as in Table 2.

phases (typically C_{18}, C≡N and phenyl) during method development in order to find the best selectivity, comparison of phases of the same class has been avoided in the past, primarily because of concerns about reproducibility from batch to batch or over a period of years.

The batch-to-batch reproducibility of HPLC silica phases has been improved considerably during the last decade. The end-capping methods or intentional alterations in the C_{18} chains (like in the case of the Supelco ABZ column) should also produce fairly reproducible results for an extended period of time if the chromatographic conditions are not too harsh.

It is worth noting that as the attempts to eliminate matrix interaction by using high purity, metal-free silica support become more and more successful, from the point of chromatographic behavior columns of this type from different manufacturers are getting closer to each other and to the ideal C_{18} phase. For the end-user these columns are practically interchangeable and may be listed as 'equivalent' in an assay method after minimal validation. As our results show, this is definitely not true for all C_{18} phases.

Table 4 contains the method validation results obtained under the final conditions. As the figures show, the method meets all common requirements for accuracy, precision and linearity. Essentially identical results generated by a second chemist demonstrated that it is also rugged.

The stability studies, in accordance with previous observations [1,7], confirmed the sensitivity of chlorpromazine to light and strong oxidants and the hydrolysis of the parabens in the presence of strong bases. The degradation products detected in the cases above were chlorpromazine sulfoxide and *p*-hydroxy benzoic acid, respectively. Both compounds elute before methylparaben without interference with any of the analytes.

Under normal conditions using low-actinic glassware, the sample and standard solutions were stable up to eight days.

The method may also be used for the determination of chlorpromazine sulfoxide in the raw material or finished product. The limit of quantitation (calculated for a peak height ten times the average peak-to-peak baseline noise) is $7 \cdot 10^{-5}$ mg ml^{-1}, well below the established USP limit for the raw material (less than 5% chlorpromazine sulfoxide in chlorpromazine hydrochloride).

4. Conclusion

The method discussed in this report provides a convenient and accurate way to analyze a compound of the phenothiazine family in the presence of paraben preservatives.

Since an endless variety of mobile phase compositions is readily available, chemists often overlook other possibilities during method development. After making the basic choices, they typically use whatever column is in sight. In cases like the one described in the present study, fine-tuning of a method by comparison of columns of the same

Table 4
Method validation results for all analytes

Validation Step	Parameter	Methylparaben	Propylparaben	Chlorpromazine	Chl. Sulfoxide	Criteria
System precision	Standard area R.S.D.	0.12%	0.52%	0.24%	0.59%	x<25
	Sample area R.S.D.	0.10%	0.30%	0.10%	0.14%	x<2%
Method precision	Sample area R.S.D.[a]	0.56%	1.12%	0.72%	0.27%	x<2%
Accuracy	Spike recovery[b]	99.9%	99.6%	100.4%	100.2%	97%<x<103%
	Recovery R.S.D.[b]	0.10%	0.45%	0.41%	0.31%	x<2%
Linearity	Correlation coeff.	0.9995	0.9995	0.9996	0.9995	x>0.999
Sample stability[c]	% change in response factors	1.74%	0.46%	0.62%	0.67%	x<2%
Stress degradation	%Recovery	>97%	>95%	>95%	n/a	x>95%

Conditions as in Table 2. All R.S.D.'s are from five injections, unless otherwise noted
[a]: five preparations, two injections each.
[b]: at 80%, 100% and 120% levels, three preparations each, two injections of each preparation.
[c]: four-day stability data for chlorpromazine sulfoxide, eight-day data for all others.

general type can be an effective tool. In the past, this kind of method development was mostly avoided due to reproducibility problems. The new specialty columns available today can provide a reproducible range of selectivity within the same class, e.g. C_{18}.

At the same time the results show that the columns where the major improvement in technology was the manufacturing of higher purity silica, show very similar characteristics. Since the matrix contribution to the overall separation is minimal, these columns are closer to the ideal C_{18} phase and therefore more interchangeable.

The stability of the specialty columns used in this application is subject to further investigation.

Acknowledgements

The authors thank Mr. T.E. Wheeler for the useful comments.

References

[1] USP XXIII, The United States Pharmacopeia, Rand McNally, Taunton, MA, 1995, pp. 353–356.
[2] G. Tamai, H. Yoshida, H. Imai, J. Chromatogr. 423 (1987) 163.
[3] J.S. Kiel, S.L. Morgan, R.K. Abramson, J. Chromatogr. 485 (1989) 485.
[4] T. Ohkubo, R. Shimoyama, K. Sugawa, J. Chromatogr. 614 (1993) 328.
[5] S.H. Curry, E.A. Brown, O.Y.-P. Hu, J.H. Perrin, J. Chromatogr. 231 (1982) 361.
[6] L.R. Snyder, J.L. Glajch, J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, 1988, p.232.
[7] Method #0742.06, Alpharma Inc., USPD, (1994).
[8] Waters Column, Special Edition (1994) 1.
[9] Alltech Bulletin #247B, October (1994) 3.
[10] T. Ascah, F. Liu, The Reporter (Supelco, Inc.). 14 (1995) 4.
[11] V.R. Meyer, LC·GC 13 (1995) 252.