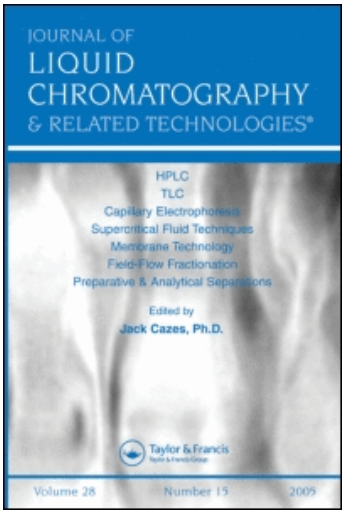

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Pentoxyverine in Cough Preparations by High Performance Liquid Chromatography

E. A. Gad-Kariem[a]; M. A. Abounassif[a]
[a] Department of Pharmaceutical Chemistry, College of Pharmacy King Saud University, Riyadh, Saudi Arabia

To cite this Article Gad-Kariem, E. A. and Abounassif, M. A.(1997) 'Determination of Pentoxyverine in Cough Preparations by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 20: 18, 3049 — 3059
To link to this Article: DOI: 10.1080/10826079708006580
URL: http://dx.doi.org/10.1080/10826079708006580

J. LIQ. CHROM. & REL. TECHNOL., 20(18), 3049–3059 (1997)

DETERMINATION OF PENTOXYVERINE IN COUGH PREPARATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

E. A. Gad-Kariem,* M. A. Abounassif

Department of Pharmaceutical Chemistry
College of Pharmacy
King Saud University
P.O. Box 2457
Riyadh-11451, Saudi Arabia.

ABSTRACT

A simple liquid chromatographic method for quantification of pentoxyverine in cough preparations is described. The LCseparation of the drug from the complex matrices of the dosage formulations was undertaken on a reversed phase 100RP-18 Lichrosphere (5 μm) column, 15 cm × 4.6 mm i.d. The mobile phase used was methanol and 25% w/w ammonia in the ratio 99.2 : 0.8, v/v isocratically at a flow rate of 1.2 mL min^{-1} with U.V. detection at 258 nm, at ambient temperature. Good percentage assay and mean added recovery results were obtained with relative standard deviations (RSD) less than 2%.

INTRODUCTION

Pentoxyverine (carbetapentane), 1-phenylcyclopentane carboxylic acid 2-(2-diethylaminoethoxy)ethyl ester, is available as the citrate or HCl salt. It is a non-narcotic antitussive with selective action on cough center.[1-4] A number

of methods have been reported for the assay of pentoxyverine citrate in bulk form,[5-7] in tablet formulation[8-15] and in syrup form.[16] The cited methods, which are all in Chinese, included flourimetric,[5] potentiometric,[6] titrimetric with different titrants,[7-12] colorimetric,[13] first-derivative U.V.-spectrophotometric,[14] a neutralisation extraction technique,[15] and a derivative spectrophotometry-damping factor matrix method.[16]

As there is no method reported for the determination of pentoxyverine in cough preparations containing it, it was deemed useful to develop an HPLC method for the routine and accurate determination of the drug in presence of various excipients, sugar bases and other combinations encountered in such preparations. The developed HPLC method was successfully applied to two cough preparations marketed in Saudi Arabia.

EXPERIMENTAL

Waters liquid chromatograph 600E, equipped with Waters-U6K Millipore Injector, Waters-486 tunable absorbance detector, and Waters-746 data module was used. The column used was stainless steel, 15 cm × 4.6 mm i.d., packed with 5 μm Lichrosphere 100RP-18 bonded material. The mobile phase was composed of methanol (HiperSolv™ BDH Chemicals Ltd., Pool-UK) and 25% w/w ammonia (Analar BDH) in the ratio 99.2 : 0.8, v/v, pumped isocratically at a flow rate of 1.2 mL min^{-1}.

Degassing of the mobile phase was carried out by purging pure helium into the solvent reservoir at a rate of 10 mL min^{-1}, U.V. setting was at 258 nm and twenty microlitre volumes were injected into the column at room temperature.

Materials and Reagents

Reference pentoxyverine citrate was kindly provided by the central laboratory in Riyadh and it was used, as received, without further treatment. Toclase® syrup (BN 94G29 MFD 07/94) containing 7.5 mg pentoxyverine hydrochloride in each 5 mL and Toclase® + expectorant oral solution (BN 94F09 MFD 06/94) containing pentoxyverine citrate 10.65 mg, terpine hydrate 15 mg, and sodium citrate 65 mg in each 5 mL, were collected from local pharmacies in Riyadh. Chlorpromazine hydrochloride (Winlab) was used as internal standard.

Standard Solutions and Samples Preparation

Reference pentoxyverine citrate stock solution

0.2000 % w/v in methanol.

Internal standard

Chlorpromazine hydrochloride stock solution 0.020% w/v in methanol was diluted with methanol to give 10 μg mL^{-1} solution.

Standard series

1-8 mLs of the reference drug stock solution were transferred into 25 mL volumetric flasks; to each flask was added 4.0 mLs of internal standard solution (10 μg mL^{-1}) and volume completed with methanol.

Triplicate injections of each dilution were made and regression analysis of concentration *vs* peak ratio of drug against internal standard was obtained.

The slope consistency of the prepared standard was checked at different days; within day (n = 5) and between day runs (n = 15) for three different concentrations at low, medium, and high levels of the standard curve were done and relative standard deviations were calculated to check for the reproducibility and precision of the method.

Sample preparation

2 mL, 3 mL, and 4 mL of each preparation were transferred into 25 mL volumetric flasks and 4.0 mL of internal standard was added; volume completed with methanol and 3 to 5 runs were done for each sample solution. Calculations for concentration were either computed from regression analysis data or from a direct comparison of sample to an equivalent standard solution.

When using the direct comparison method the following formula can be adopted for calculation:

$$\text{C mgs/5 ml as citrate} = \frac{A}{B} \times C\,(mg) \times \frac{5}{V}$$

$$\text{or as HCl} = \frac{A}{B} \times C\ (mg) \times \frac{5}{V} \times 0.70377$$

where A = sample peak ratio, B = standard peak ratio, C = mgs of standard in the volume of standard used and V = volume of cough preparation taken.

Recovery Experiment

To 2 mL of either sample was added 2 mL of standard solution (0.200 %, w/v) and 4.0 mL of internal standard (10 μg mL^{-1}) and volume completed to 25 mL with methanol. Triplicate injections were made for each solution. On the other hand, separate 2 mL volumes of each sample and separate 2 mL standard solution (0.200% w/v) were transferred into 25 mL volumetric flasks and to each flask was added 4.0 mL of internal standard (10 μg mL^{-1}), volume completed with methanol and triplicate injections were made for each solution; recovery was calculated as follows:

$$\frac{P_{(ad)} - P_{(sp)}}{P_{(st)}} \times 100$$

where $P_{(ad)}$ = peak ratio for added solution, $P_{(sp)}$ peak ratio for sample solution and $P_{(st)}$ peak ratio for standard solution.

RESULTS AND DISCUSSION

Different mobile phases and columns were tried for the separation and quantification of pentoxyverine in Toclase® syrup and Toclase® + expectorant oral solution. Mobile phases containing water were found to cause delayed and badly tailing peaks for the drug with the columns tried. No elution occurred when using acetonitrile alone or in combination. Methanol, on the other hand, was promising and needed ammonia to improve peak shape and retention time. This was found satisfactory at a ratio of 99.2 : 0.8, v/v methanol : ammonia; where the capacity factor and tailing factor of the eluting peak were compared for the different columns used. With phenyl column capacity factor (K_1) was about 0.6 (fast). For C18 Bondapak K_1 value was about 2 but with tailing factor exceeding 2.0. Lichrosphere 100RP-18 (5 μm) 12.5 cm × 4.0 mm, i.d. and Lichrosphere 100RP-18 (5 μm) 15 cm × 4.6 i.d. were the best to use with K_1 values of about 1.35 and 1.95, respectively at a flow rate of 1.2 mL min^{-1}; however, for the syrup and oral solution only Lichrosphere 15 cm × 4.6 mm, i.d. resolved the drug from the sugar bases and other excipients encountered in

Table 1

Comparative Study of Chromatograms of Solutions in Water and in Methanol*

Solvent	Solvent Peak	Tailing Factor	Capacity Factor (K_1)	Resolution Between Drug and Internal Standard
Water	Negative	Drug ≈ 1.90 Internal Standard ≈ 1.5	Drug 2.06 Internal Standard ≈ 2.91	2.15
Methanol	-----	Drug ≈ 1.6 Internal Standard ≈ 1.5	Drug ≈ 1.95 Internal Standard ≈ 2.78	2.33

* Refer to Figure 1 (a,b).

these preparations. The lowest tailing factor obtained was about 1.6 which is considered within acceptable practically found values (≤ 1.6).[17] Internal standard was used, so as to minimize possible errors.[17] Several drugs were tested for selection of a suitable internal standard and testing possible interference as indicative for specificity. Chlorpromazine hydrochloride (K_1 = 2.78) was found to be a suitable internal standard for this study. Also during the first trials to obtain optimal conditions for this study, solutions of reference drug, internal standard and samples were prepared and diluted with water as a cheap solvent, before injecting onto the column. The obtained chromatograms were compared with those obtained for similar solutions prepared in methanol.

Table 1 summarizes the comparative study which indicated better conditions when methanol was used throughout the assay. Also, a peak response of about 25% increase in height was obtained with methanol solutions Fig. 1 (a, b). The slight difference in resolution (2.33 and 2.15) was significant since it gave more reproducible peak-ratios. Sugar in Toclase® syrup tend to crystallize slowly from methanol solutions on standing for about three hours or on vigorous shaking of the solution without any effect on the single peak areas of the drug or internal standard and hence on the peak ratio. Fig. 2 (a, b) shows typical chromatograms for both preparations.

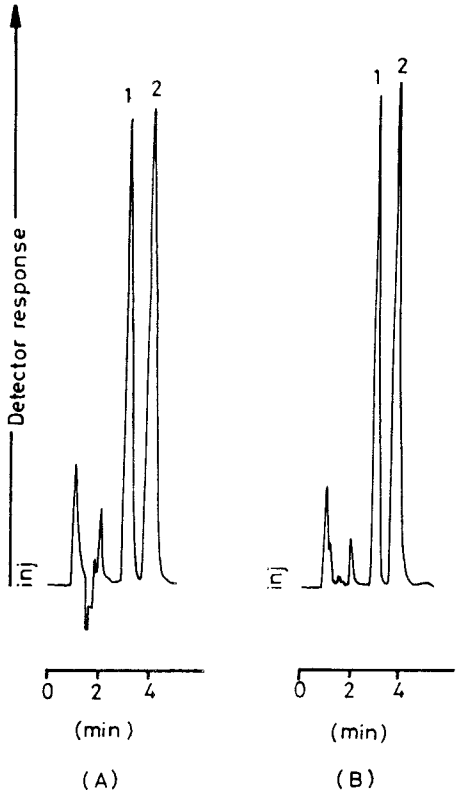

Figure 1. a) Typical chromatogram of injected aqueous solutions of pentoxyverine citrate (1) (0.04% w/v) and chlorpromazine HCl (internal standard) (2) (2×10^{-4}% w/v). b) Typical chromatogram of injected methanolic solutions of pentoxyverine citrate (1) (0.032%, w/v) and chlorpromazine HCl (internal standard) (2) (1.6×10^{-4} %, w/v).

Good system suitability and column efficiency were reflected by the acceptable data obtained for capacity factor (K_1), resolution, tailing factor, number of theoretical plates, height equivalent to theoretical plate (HETP)[17,18] and the relative standard deviation as in Table 2.

Pentoxyverine weekly absorbs in U.V. region as it contains only a phenyl group as the effective chromofore with typical benzenoid absorption at 262 nm, 258 (λ_{max}) and 254 nm. The calculated molar absorptivity has a log $\epsilon$of 2.27 in

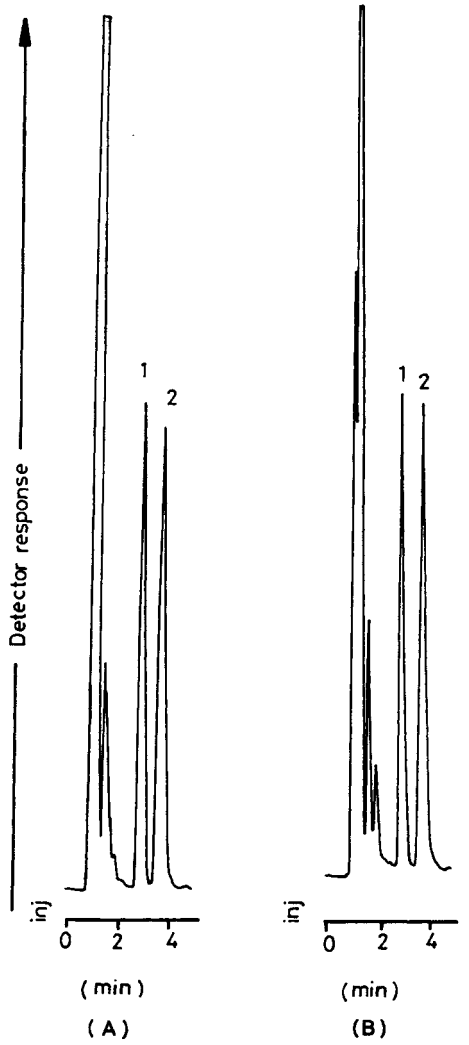

Figure 2. a) Typical chromatogram of resolved pentoxyverine HCl (1) (0.03408 %, w/v, citrate) and chlorpromazine HCl (internal standard) (2) (1.6×10^{-4}, w/v) from Tolcase® syrup. b) Typical chromatogram of resolved pentoxyverine citrate (1) (0.03408 %, w/v) and chlorpromazine HCl (internal standard) (2) (1.6×10^{-4} %, w/v) from Tolcase® + expectorant oral solution.

Table 2

Parameters for Resolved Pentoxyverine Citrate and Chlorpormazine Hydrochloride (Internal Standard)

Parameter	Pentoxyverine Citrate	Chlorpromazine Hydrochloride
Detection wavelength (λ_{max} nm)	258	
Flow rate (mL min^{-1})	1.2	
Capacity factor (K')	1.95	2.78
Resolution (Rs)	2.33	
Tailing factor	≈ 1.65	≈ 1.50
Number of theoretical plates N/m	20408	
HETP mm	0.049	
Relative standard deviation (RSD%)	< 2.0	
Analysis time	4.5 min	

methanol and 2.24 in water at 258 nm. With this low absorptivity pentoxyverine is considered a good candidate for the highly sensitive HPLC U.V. detectors: the absorption was monitored at 258 nm (λ_{max}) so as to get maximum sensitivity.[17]

Regression analysis of the calibration curve (conc. *vs* peak-ratio) indicated a linear relationship between peak-ratio (Y) and conc. ($\mu g\ mL^{-1}$) for pentoxyverine citrate (range 80 $\mu g\ mL^{-1}$ to 640 $\mu g\ mL^{-1}$).

$$Y = 1.2 \times 10^{-3} + 2.2775 \times 10^{-3}\ C$$

with a correlation coefficient, r = 0.9999. The detection limit was 8 $\mu g\ mL^{-1}$ at a signal to noise ratio of 3:1.

The reproducibility and precision of the method was assessed by the follow up of within day, between day data for low, medium, and high concentrations within the standard curve, and also by the follow up of the slope consistency for data of standard curve injected for four days. RSDs in all three cases were less than 2% indicating good reproducibility and precision of the method (Table 3). Table 4 collects the obtained results of assay of pentoxyverine in Toclase® syrup and Toclase® expectorant oral solution, as well as the recovery testing of added amount of pentoxyverine citrate to the two preparations. The obtained results reflected excellent precision and accuracy.

Table 3

Reproducibility and Precision as Evaluated by RSD% for Within-Day, Between-Day and Slope Consistency

Concentration ($\mu g\ mL^{-1}$)	Within-day RSD %	Between-day (n=15) RSD %	Slope Consistency $\bar{x}$ B ± SD (n=20) RSD %
Low (80)	1.38	1.5	$2.2798 \times 10^{-3} \pm$
Medium (400)	1.14	1.00	1.8807×10^{-5}
High (640)	0.92	0.64	0.82

n: Number of independent determinations.
RSD: Relative standard deviation.
$\bar{x}$ B: Average peak ratio per unit concentration ($\mu g\ mL^{-1}$).

Table 4

Assay and Added Recovery of Pentoxyverine in Pharmaceutical Formulations

Preparation	Volume Taken (mLs)	Claimed Content/ Volume Taken (mg)	n	Found Average for (n) Expts	Assay (%)	Added Recovery X± SD CV (n)
Tolcase®	2	3.0	3	3.01	100.70	
syrup	3	4.5	5	4.508	100.18	
(pentoxyverine	4	6	5	6.146	102.44	100.82 ± 1.25
HCl)						1.24 (3)
		Pooled Assay Result (n = 13) X ± SD 101.17 ± 1.39				
Tolcase® +	2	4.26	3	4.1995	98.58	
expectorant	3	6.39	5	6.296	98.53	
(pentoxyverine	4	8.52	5	8.36	98.18	100.43 ± 0.78
HCl)						0.78 (3)
		Pooled Assay Result (n = 13_) X ± SD 98.38 ± 0.83				

X = Arithmetic mean. SD = Standard deviation. CV = Coefficient of variation. n = no of independent determinations.

Pentoxyverine is marketed as the hydrochloride salt in Toclase® syrup and as the citrate in Toclase® + expectorant oral solution. In this study, both were determined in terms of the citrate using reference pentoxyverine citrate and an adopted formula given for the calculation of content per 5 mL preparation either as the citrate or as the hydrochloride (see Experimental).

Although the method was applied to Toclase® syrup and Tolcase® + expectorant oral solution only, it is expected to be applicable for Tolcase® tablets which were not available in Riyadh local pharmacies.

It's worth noting that all cited methods in the literature were for the drug in bulk form[5-7] and in tablet formulation[8-15] and only one method was for a syrup form with combination different from those of the formulations[16] studied. Also, all the cited methods were in Chinese, with the exception of the potentiometric method,[6] used for the determination of the drug in bulk form.

CONCLUSION

The HPLC method described in this study is the only method for the separation and quantification of pentoxyverine in the formulations of cough preparation studied. The sensitivity, reproducibility, simplicity, and short analysis time (4.5 min) of the method makes it valuable in the routine analysis of pentoxyverine in its available formulations.

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Received December 22, 1996
Accepted March 6, 1997
Manuscript 4364