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Determination of diphenylpyraline in plasma and urine by high-performance liquid chromatography

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Abstract

A rapid, reliable and specific reversed-phase high-performance liquid chromatographic procedure is described for the determination of diphenylpyraline hydrochloride at nanogram concentrations in plasma and urine. After extraction of the drug with *n*-pentane–2-propanol (50:1) from alkalinized samples, the organic extract was evaporated to dryness, reconstituted with methanol and chromatographed using a 5-μm Asahipak ODP-50 C_{18} column with UV detection at 254 nm. The elution time for diphenylpyraline was 7.9 min. The overall recovery of diphenylpyraline from spiked plasma and urine samples at concentrations ranging from 53 to 740 ng/ml were 94.65% and 92.29%, respectively. Linearity and precision data for plasma and urine standards after extraction were acceptable. The limit of detection was 15 ng/ml for both plasma and urine samples at 0.002 AUFS.

Keywords: Diphenylpyraline

1. Introduction

Antihistamines are among the most widely used drugs and are readily available by prescription and over the counter as single-entity preparations and in mixtures. In general, they are effective in the management of allergic disorders caused by the release of histamine [1]. Diphenylpyraline (4-benzhydryloxy-1-methylpiperidine) hydrochloride is an antihistaminic drug and also causes mild sedation. It has the general properties and uses of antihistamines [2,3].

Diphenylpyraline combined with other drugs has been separated by thin-layer chromatography (TLC) and determined with UV spectrophotometric detection [4]. The method involves multiple extraction steps, followed by TLC separation before the final determination of the isolated components by spectrophotometry. The whole procedure is considered slow, laborious and time consuming.

The drug has been used as an internal standard in the determination of twelve antihistamines and some related compounds in pharmaceutical preparations at the microgram level by gas chromatography [5]. It has also been determined in combination with xanthines in pharmaceutical formulations by gas–liquid chromatography (GLC) [6]. However, the GLC method involves

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the derivatization of the drugs. Moreover, the overlapping of the diphenylpyraline peak by a precursor peak from the reaction mixture would have affected the accuracy of its measurement. Sidhu et al. [7] and Roos and Lau-Cam [8] have reported general HPLC methods for the analysis of pharmaceutical dosage forms and for the separation of acidic, basic and neutral drugs, including diphenylpyraline, respectively. The chromatographic system in one of the HPLC methods [7,9] included acetonitrile in potassium hydrogenphosphate, but our preliminary studies indicated that the use of potassium phosphate buffer resulted in the formation of precipitates in the mobile phase. Therefore, acetate buffer that was easily miscible with the organic solvent and improving peak symmetry was used. Since the literature on the determination of diphenylpyraline is limited, especially in biological fluids, it was considered necessary to develop and evaluate a simple, specific and applicable HPLC method for its determination in plasma and urine samples.

2. Experimental

2.1. Chemicals and solutions

Diphenylpyraline hydrochloride and pimethixene bimalate were obtained from Sigma (St. Louis, MO, USA) and used without further purification. HPLC-grade methanol and water were used throughout. All other chemicals were of analytical-reagent grade and were used as received.

Stock solutions were prepared by accurately weighing the appropriate amounts of diphenylpyraline and pimethixene and dissolving each separately in methanol in a 50-ml volumetric flask to prepare 1 mg/ml stock standard solutions. Working standard solutions of diphenylpyraline were prepared from the stock solution by sequential dilutions with methanol to give concentrations of 53, 106, 211, 317, 422, 528, 634, 739 ng/ml, containing 400 ng/ml of pimethixene as internal standard.

2.2. Instrumentation

The HPLC apparatus consisted of a Shimadzu Series LC-6A with two high-pressure pumps and an SPD-6AV UV spectrophotometric detector controlled by an SCL-6B system programmer module and was operated at 254 nm. Samples were injected using a Rheodyne Model 7167 injection valve with a 20-μl loop, and chromatograms were recorded with Chromatopac CR-6A integrator at a speed of 1 cm/min. All components were obtained from Shimadzu (Kyoto, Japan).

2.3. Chromatographic conditions

The chromatographic separation was performed on a C_{18} polymeric reversed-phase (5-μm) column (Asahipak ODP-50; 150 mm $\times$ 6.0 mm I.D.) (Asahi, Japan). The mobile phase was 0.05 *M* acetate buffer–methanol (62:38). The apparent pH was adjusted to 3.5 using glacial acetic acid after mixing appropriate volumes of methanol and aqueous ammonium acetate. The column was equilibrated with the eluting solvent by pumping the mobile phase at a rate of 0.3 ml/min and degassed by slowly bubbling helium gas overnight. The flow-rate was set at 1.4 ml/min during analysis and detection was performed at 254 nm with a sensitivity of 0.002 AUFS.

2.4. Extraction procedure

To 1 ml of plasma or human urine samples, in 12-ml conical glass centrifuge tubes, were added 1-ml volumes of the eight standard solutions at concentrations ranging from 53 to 739 ng/ml and containing a fixed amount (400 ng/ml) of pimethixene, used as the internal standard. Then 0.5 ml of 3 *M* NaOH solution were added to the solution and mixed briefly to make it alkaline. After the addition of 3 ml of *n*-pentane–2-propanol (50:1), the mixture was vortex mixed at high speed for 2 min and centrifuged at 2000 rpm for 5 min in order to separate the two phases clearly. The upper organic phase was transferred into another tube and the extraction step was repeated. The combined organic phases (2 $\times$ 3)

ml were evaporated to dryness at ambient temperature under a stream of nitrogen. The residue was dissolved by addition of 1 ml of methanol, and subsequently an aliquot of 2 μl was injected on to the HPLC column.

3. Results and discussion

Fast and reproducible separation of the analytes was achieved using a C_{18} reversed-phase column, eluted isocratically with acetate buffer of constant ionic strength 0.05 *M*–methanol (62:38). The use of the acetate buffer increased the sharpness of the chromatographic peaks, thus improving the performance of the column for the separation of diphenylpyraline and pimethixene. Fig. 1 shows representative chromatograms of blank extracts from urine and plasma and extracts of diphenylpyraline together with pimethixene at nanogram levels used as an internal standard from plasma and urine. The peaks of both pimethixene and diphenylpyraline were well resolved and their symmetry was satisfactory. The retention times were 7.90 min for diphenylpyraline and 13.40 min for pimethixene, and their resolution factor was 3.67. The use of the mixed organic solvent system of *n*-pentane–2-propanol was suitable for the quantitative extraction of the drug from basified plasma and urine. Moreover, 2-propanol retards the absorption of this basic drug on the glass surface during extraction. This observation has been described previously for the assay of the antipsychotic agent trifluoperazine in plasma [10]. The calibration graphs were linear over the concentration range 53–740 ng/ml ($r > 0.9993$) for both plasma and urine samples. The recovery and accuracy of the assay was assessed by using a diphenylpyraline calibration graph of the peak-height ratio of diphenylpyraline to the internal standard after direct injection of methanolic

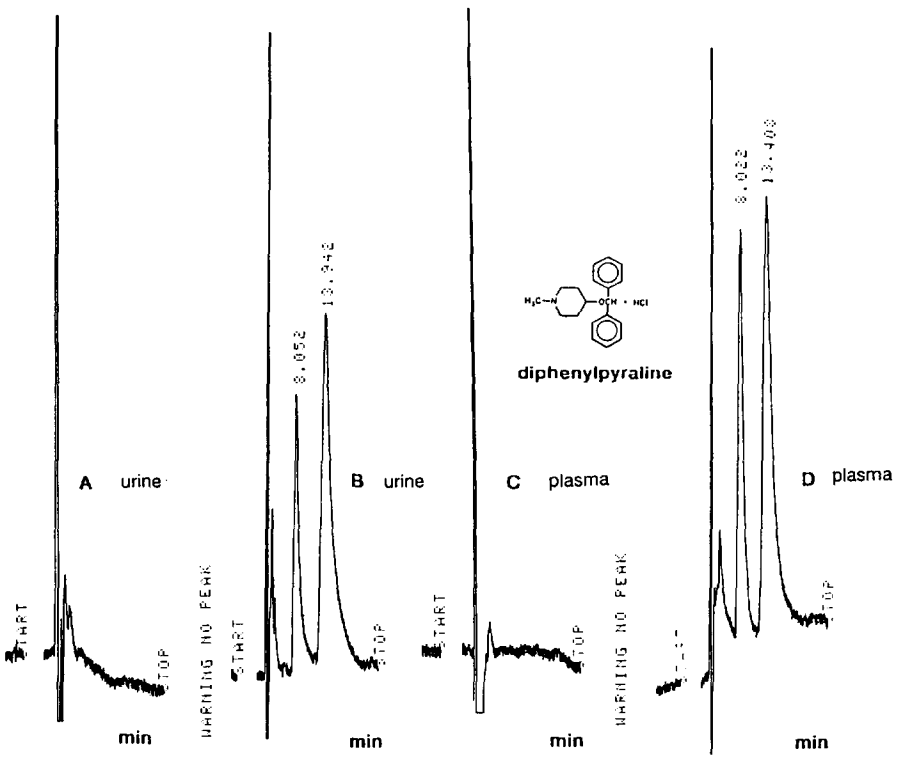

Fig. 1. Representative chromatograms of blank (A) urine and (C) plasma extracts and (B) urine and (D) plasma extracts that were spiked with 317 ng/ml diphenylpyraline (t_R = 7.90 min) and with 400 ng/ml pimethixene (internal standard) (t_R = 13.40 min).

Table 1
Standard calibration and recovery data for diphenylpyraline in spiked plasma ($n = 4$)

Added (ng/ml)	Mean peak-height ratio	Found (mean ± S.D.) (ng/ml)	C.V. (%)	Recovery (%)
53	0.257	49.93 ± 0.58	1.21	90.43
106	0.529	98.04 ± 1.18	1.20	92.49
211	1.086	200.65 ± 2.29	1.14	95.10
317	1.600	295.34 ± 3.65	1.24	93.17
422	2.143	395.37 ± 4.28	1.08	93.69
528	2.714	500.56 ± 5.32	1.06	94.80
634	3.257	600.59 ± 7.03	1.17	94.73
739	3.771	695.28 ± 7.54	1.08	94.08

$y = 0.005x - 0.014$ ($r = 0.9999$). Average recovery = 93.56%. Average C.V. = 1.15%.

solutions containing known amounts of diphenylpyralines ranging between 53 to 739 ng/ml versus concentration and, by using the characteristics of this calibration graph, the amount of the drug recovered after extracting spiked plasma and urine samples containing equivalent amounts of the drug was calculated from the standard calibration graph. At each of the eight diphenylpyraline concentrations used, four replicate samples were measured. Results of the standard calibration and recovery data are given in Tables 1 and 2, respectively.

The overall recovery of the drug was calculated by plotting a standard calibration graph of the "added" versus the "found" concentrations of diphenylpyraline in both plasma and urine samples, which gave the linear relationships $y = 0.9465x - 1.91$ ($r = 0.9999$) for plasma and $y = 0.9229x + 1.51$ ($r = 0.9992$) for urine. Therefore, the slopes (0.9465 for plasma and 0.9229 for urine) of these regression lines were used as estimates of the overall recovery for diphenylpyraline hydrochloride, 94.65% for plasma and 92.29% for urine samples [11]. The accuracy and precision of the method were determined by spiking plasma and urine samples with diphenylpyraline hydrochloride standards at two concentration levels (106 and 422 ng/ml), each containing 400 ng/ml of pimethixene as internal standard.

Table 2
Standard calibration and recovery data for diphenylpyraline in spiked urine ($n = 4$)

Added (ng/ml)	Mean peak-height ratio	Found (mean ± S.D.) (ng/ml)	C.V. (%)	Recovery (%)
53	0.254	47.38 ± 0.64	1.35	89.40
106	0.521	98.04 ± 1.25	1.28	92.49
211	1.056	195.12 ± 2.63	1.35	92.47
317	1.549	285.94 ± 4.03	1.41	90.20
422	2.211	407.90 ± 5.26	1.29	96.66
528	2.690	496.14 ± 6.91	1.39	93.97
634	3.183	586.96 ± 7.14	1.22	92.73
739	3.648	672.62 ± 7.86	1.17	91.02

$y = 0.005x + 0.002$ ($r = 0.9993$). Average recovery = 92.35%. Average C.V. = 1.31%.

Extraction and HPLC determinations were carried out as described above. The within-day accuracy and precision for each concentration level were obtained from four analyses of samples of the same concentration and the overall accuracy and precision for each concentration level were obtained from twelve analyses of samples of the same concentration on three different days (4×3) within 1 month. Therefore, for the assessment of the overall precision within 1 month at two concentration levels, 24 plasma and urine samples each were treated. The results are presented in Tables 3 and 4.

The detection limit of the assay, defined as the minimum drug concentration to produce twice the signal-to-noise ratio at 0.002 AUFS, was found to be ca. 15 ng/ml for both plasma and urine samples.

4. Conclusion

The chromatographic system described here has been shown to be applicable to the determination of diphenylpyraline at nanogram levels in plasma and urine. The method is fast, reproducible, specific and sensitive and could be useful in further investigations on the pharmacokinetics of diphenylpyraline in clinical studies and also in routine analysis.

Table 3
Accuracy and precision of the determination of diphenylpyraline in spiked plasma ($n = 4$)

Spiked amount (ng/ml)	Day	Accuracy (mean found concentration) (ng/ml)	Precision		Overall (mean concentration ± S.D.) (ng/ml)
			S.D. (ng/ml)	C.V. (%)	
106	1	98.40	1.67	1.70	98.16 ± 1.85
	2	97.93	2.39	2.40	
	3	98.15	1.20	1.22	
422	1	399.08	4.00	1.00	397.89 ± 3.40
	2	393.55	3.81	0.97	
	3	401.03	4.25	1.06	

Table 4
Accuracy and precision of the determination of diphenylpyraline in spiked urine ($n = 4$)

Spiked amount (ng/ml)	Day	Accuracy (mean found concentration) (ng/ml)	Precision		Overall (mean concentration ± S.D.) (ng/ml)
			S.D. (ng/ml)	C.V. (%)	
106	1	96.90	1.43	1.48	97.39 ± 1.44
	2	98.21	1.27	1.29	
	3	97.05	1.56	1.61	
422	1	400.0	4.53	1.13	400.80 ± 4.70
	2	403.78	4.66	1.15	
	3	398.62	4.90	1.22	

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