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Short communication

Development and validation of LC–MS/MS method for the determination of cyproheptadine in several pharmaceutical syrup formulations

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ABSTRACT

A rapid and sensitive liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method was developed and validated for the qualitative and quantitative assay of cyproheptadine (CP) in pharmaceutical samples. Diphenylpyraline hydrochloride (DPP) was used as an internal standard (IS). Two multiple reaction-monitoring (MRM) transitions for each analyte were observed: 288.1/96.1 and 288.1/191.2 for CP and 282.1/167.2 and 282.1/116.3 for DPP. The retention time of the drug was 7.29 min. The analytical method was successfully validated for linearity (1–100 ng/ml), intra-day precision, inter-day precision, and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) were 0.86 and 0.98 ng/ml, respectively. The proposed method was applied to analyse the cyproheptadine content from seven different syrup formulations.

1. Introduction

Cyproheptadine hydrochloride (CP, Fig. 1) (4-(5H-dibenzo[a,d]-cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride) is an antihistaminic, antiserotonergic agent, known to have inhibitory activities for L-type calcium channels [1].

Previously, CP was evaluated in patients for the treatment of anorexia, migraines, and atopic dermatitis [2–4]. Investigations with animals suggested that CP has obvious therapeutic effects on traumatic brain oedema [5], can protect spinal cord ischemic injury [6], increase cerebral blood flow causing cerebral vasodilation [7], and can induce anti-shock effects [8]. More recently, CP was identified as the lead novel therapeutic agent for the treatment of cancer, as an inhibitor of D-cyclin expression, leading to induced cell death and delayed tumour growth in mouse models [9].

In veterinary medicine, CP is indicated to treat anorexia, weight loss, lack of growth, convalescence, and muscular weakness; it is additionally used as a non-specific tonic. In all cases, CP use is specifically not allowed for animals destined for food production. However, we are suspicious about the illegal use of CP in meat production, because the existence of a black market for chemical cocktails used illegally for growth promotion in food-producing animals is well known. Furthermore, CP is included on the list of prohibited ingredients in cosmetics by the Japanese Pharmaceutical Affairs Act [10].

On other hand, special attention has recently been paid to CP in the literature, because significant structural similarities between CP and tricyclic antidepressants (TCAs) have induced false positive results in TCAs analysis. Cyproheptadine has a 3-ringed molecular structure resembling the TCAs, and can affect the assays to detect the presence of these antidepressants, as reported in a previous work [11].

This has been of interest not only in the fields of clinical toxicology and pharmacology, but also in forensics, because TCAs are often involved in intoxications [12–14].

In consulting the scientific literature, several analytical methods have been reported for the detection of CP, but in all of the cases, attention focused on human samples or laboratory animals. In these reports, gas–liquid chromatography [15], high-performance liquid chromatography with photodiode-array detection [16,17] and only a few MS detection [18–20] methods were employed. Methods for the determination of CP in pharmaceutical preparations were reported previously, based on their reaction with ammonium molybdate [21], using a stability-indicating high-performance liquid chromatographic assay [22], electrochemistry

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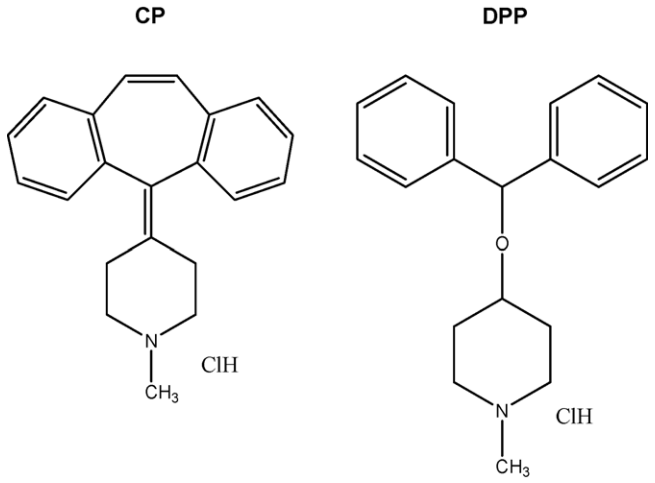

Fig. 1. Chemical structures of cyproheptadine hydrochloride (CP) and diphenylpyraline hydrochloride (DPP).

[23] or by high-performance liquid chromatography (HPLC) and chemometric methods [24].

The work presented here describes a simple method for the detection and quantification of CP in several pharmaceutical syrups, using a sensitive and specific LC–MS/MS method that is suitable for detection in the parts per billion (ppb) range.

2. Experimental

2.1. Chemicals

Cyproheptadine hydrochloride, the internal standard (IS) diphenylpyraline hydrochloride (DPP) (Fig. 1), nandrolone, boldenone, trenbolone, dexamethasone, betamethasone, flumethasone, prednisolone, triancinolone, topazole, mercaptobenzimidazole, salbutamol, clenbuterol and zilpaterol were supplied by the Sigma Company (St. Louis, MO, USA). Acetonitrile was purchased from Scharlau Chemie (Sentmenat, Barcelona, Spain). Formic acid was purchased from Acros Organics (Geel, Belgium). Anhydrous (100%) methanol and acetic acid (glacial) were supplied by Merck (Darmstadt, Germany). All chemicals and solutions were of analytical reagent grade. A Milli-Q Gradient A10 water purification system from Millipore (Bedford, MA, USA) was used.

2.2. Solutions

2.2.1. Preparation of standard solutions

Cyproheptadine hydrochloride and DPP reference standard (100 mg) were transferred to a 100-ml volumetric flask and dissolved by sonication in methanol (final concentration of 1 mg/ml). These solutions were kept at 4 °C, in the dark for no longer than 1 month. From this solution, CP standard working solutions of lower concentrations (1, 5, 10, 25, 50, 75 and 100 ng/ml) were freshly prepared by an appropriate dilution in with 10 ml water. The IS solution was similarly prepared to a final concentration of 10 ng/ml.

Table 1
Operating conditions for the chromatography separation.

Step	Time (min)	Flow rate (μl/min)	Solvent A[a] (water)	Solvent B[a] (acetonitrile)
1	1	300	90	10
2	3	300	45	55
3	6	300	55	45
4	8	300	90	10
5	10	300	90	10
6 (equilibration)	10	300	90	10

[a] Expressed in %, both with 0.1% formic acid.

2.2.2. Preparation of quality control samples

A synthetic pharmaceutical sample free of CP was prepared by dissolving 100 mg of carnitine, lysine chlorhydrate, vitamin B_{12}, sucrose, anabolic steroids (nandrolone, boldenone and trenbolone), corticosteroids (dexamethasone, betamethasone, flumethasone, prednisolone and triancinolone), thyreostats (topazole and mercaptobenzimidazole) and β-agonist (salbutamol, clenbuterol and zilpaterol) into a 100-ml volumetric flask. The solution was sonicated for 10 min with 10 ml of methanol, and the volume was made up to 100 ml with water.

Quality control (QC) samples were prepared daily by spiking synthetic pharmaceutical samples with the required volume of one of the working solutions mentioned above, to produce final concentrations equivalent to 1 ng/ml (low level), 25 ng/ml (middle level) and 100 ng/ml (high level) of CP.

2.2.3. Preparation of samples

Pharmaceutical syrup samples (Table 4) were shaken vigorously for 1 min and sonicated for 5 min. Next, a sample equivalent to 1 ml of the pharmaceutical dosage form was accurately measured, transferred to a 100-ml volumetric flask and sonicated for 10 min with 50 ml of methanol. Finally, the volume was made up to 100 ml with water. A portion of the solution was used to prepare the other diluted solutions in water, spiked with IS at a concentration of 10 ng/ml before analysis.

2.3. Liquid chromatography tandem mass spectrometric analysis

Separations were performed on an 1100 series LC system consisting of a quaternary pump, degasser and autosampler from Agilent Technologies (Minnesota, USA). A hybrid triple quadrupole linear trap Q-Trap 2000 mass spectrometer with the Ion Source Turbo Spray from Applied Biosystems MSD Sciex (Toronto, Canada) was used. Nitrogen produced by a high-purity nitrogen generator (PEAK Scientific Instruments Ltd., Chicago, IL) was used as the curtain, nebulizer and collision gas. The unit mass resolution was set in both the mass-resolving quadrupoles Q1 and Q3. The mobile phase was water mixed in gradient mode with acetonitrile, each with 0.1% formic acid (Table 1).

The flow rate of the mobile phase was 300 μl/min. A Synergi Fusion-RP (150 mm × 2 mm) 4-μm column and a guard column both from Phenomenex (Torrance, CA, USA) were used, and the injected volume in the column was 10 μl.

Table 2
Mass spectrometer parameters and monitorised transitions (RT, retention time; DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy) for cyproheptadine (CP) and diphenylpyraline hydrochloride (DPP).

Compound	RT	Q1 mass (amu)	Q3 mass (amu)	Dwell (ms)	DP	EP	CEP	CE
CP	7.29	288.10 288.10	96.10 191.20	150	46	4.5	14	41
DPP	7.13	282.11 282.11	167.20 116.30	150	46	9.5	26	75