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Detection of meclofenoxate and its degradation products Dimethylaminoethanol and *p*-chloro-phenoxyacetic acid

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1. Introduction

Since 2006 the stimulant meclofenoxate (2-dimethylaminoethyl 4-chlorophenoxyacetate) is itemised on the list of prohibited substances of the WADA. Thus screening for meclofenoxate is obligatory for accredited doping control laboratories. The psychostimulant meclofenoxate was used mainly for the treatment of mental changes in the elderly [1]. Owing to its poor stability meclofenoxate is not marketed as a drug today, but it is still available as a so-called "smart drug" for anti-aging purposes via the internet. The substance is known to be degraded rapidly to *p*-chlorophenoxyacetic acid (4-CPA) and dimethylaminoethanol (DMAE) under humid conditions, e. g. in human plasma [2-4]. As a herbicide and growth regulator for plants the degradation product 4-CPA finds additional application. The herbicide is considered to be absorbed completely from an oral dose and is eliminated rapidly unchanged in the urine [5]. Dimethylaminoethanol as a nootropic substance is naturally produced in the human brain and is available as a nutritional supplement [6].

A method based on LC-APCI-MS/MS was developed enabling the detection of meclofenoxate and its degradation products 4-CPA and DMAE in human urine. The method was used to analyse post administration urine samples as well as 3000 routine doping control samples.

2. Experimental

Reference materials

Dimethylaminoethanol (99.5 %) was purchased from Sigma (Steinheim, Germany), Helfergin™ (meclofenoxate) was obtained from Promonta (Hamburg, Germany) and 4-CPA was synthesised in our laboratory.

Sample Preparation

Urine samples were prepared utilizing the established screening procedure for diuretics [7]. Briefly, after solid phase extraction (Serdolit PAD I), washing (water), elution (methanol) and evaporation to dryness the samples were reconstituted in 200 µl of a mixture of ammonium acetate buffer (5 mmol/L, 0.1 % glacial acid) and acetonitrile (4:1, v:v).

LC-MS/MS

All analyses were performed on an Agilent 1100 liquid chromatograph (Waldbronn, Germany) interfaced to an Applied Biosystems API 2000™ triple quadrupole mass spectrometer (Darmstadt, Germany). Product ion spectra of the analytes were generated via direct infusion of the reference compounds after positive (meclofenoxate, DMAE), and after negative (4-CPA) ionisation utilizing an electrospray ionisation interface (ESI) followed by collisionally activated dissociation (CAD) with nitrogen as collision gas.

The LC was equipped with a Macherey-Nagel Nucleodor C18 Pyramid column (length 70 mm; inner diameter 4 mm, particle size 5 µm). For chromatographic separation the eluents A: ammonium acetate buffer (5 mmol/L, 0.1 % glacial acid) and B: acetonitrile were used with a gradient from 0 % B to 100 % B within 6 min. The flow rate was set to 0.8 mL/min with a static post-column split of 1:5.

For routine analyses ionisation was accomplished by means of APCI with an interface temperature of 400 ºC switching from positive to negative polarisation after 3.2 min. In the multiple reaction monitoring mode of the instrument DMAE was detected with the ion transitions: *m/z* 90→72 (CE=17 eV), *m/z* 90→70 (CE=23 eV) and *m/z* 90→57 (CE=27 eV) meclofenoxate with *m/z* 258→213 (CE=23 eV), *m/z* 258→111 (CE=55 eV) and *m/z* 258→72 (CE=29 eV) and 4-CPA with the ion transitions: *m/z* 185→127 (CE= -18 eV); *m/z* 185→91 (CE = -38 eV) and *m/z* 185→111 (CE= -18 eV). Parameters such as declustering potential, focusing potential and collision energies were optimised for maximum abundance of each ion transition of the analytes. Nitrogen was used as collision gas at a collision cell pressure of 3.7×10^{-3} Pa.

3. Results

LC-MS/MS Analyses

The mass spectrometric behaviour of the drug meclofenoxate and commercially available DMAE were studied after positive, and of synthesised 4-CPA after negative electrospray ionisation (ESI) followed CAD. The resulting product ion spectra of the protonated or deprotonated ions of meclofenoxate ($[M+H]^+$, *m/z* 258), DMAE ($[M+H]^+$, *m/z* 90) and 4-CPA ($[M-H]^-$, *m/z* 185) are depicted in Figures 1-3.

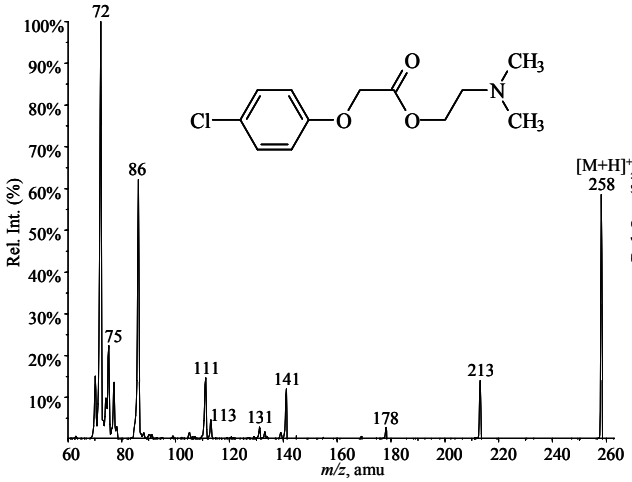

Figure 1: ESI product ion spectrum of the protonated molecule of meclofenoxate (^{35}Cl, Mw 257)

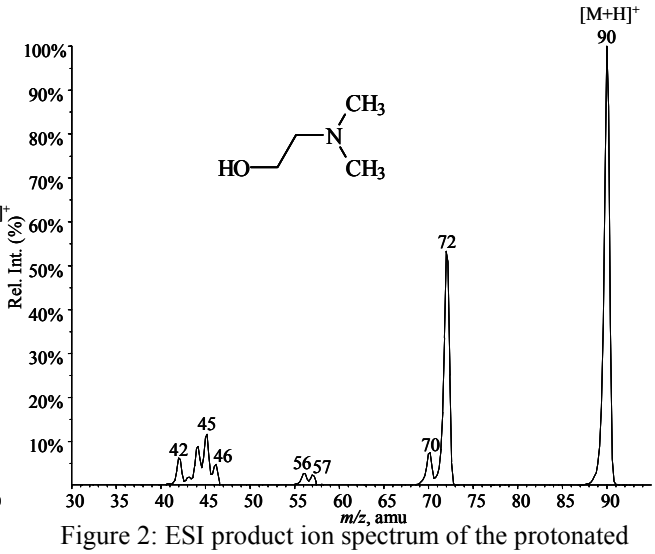

Figure 2: ESI product ion spectrum of the protonated molecule of DMAE (Mw 89)

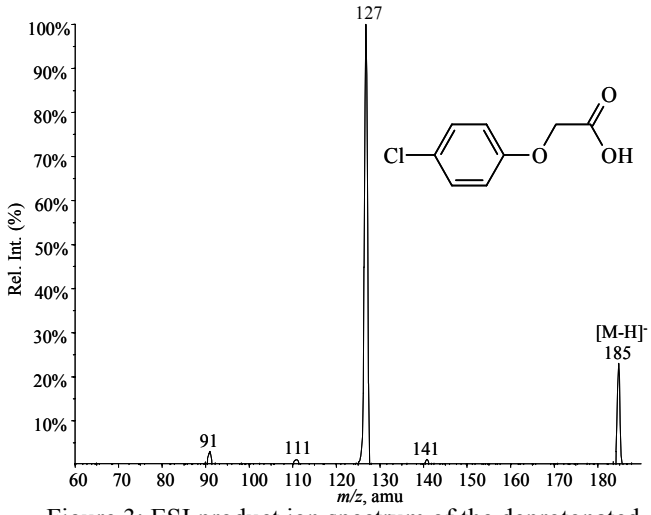

Figure 3: ESI product ion spectrum of the deprotonated molecule of 4-CPA (^{35}Cl, Mw 186)

Meclofenoxate is decomposed rapidly to DMAE and 4-CPA, e.g. in human plasma, gastric or intestinal media as well in the pure solid [2-4]. For screening purposes both degradation

products were implemented into the existing screening method for diuretics. The analyses of 3000 doping control samples provides frequent detection of 4-CPA in approx. 5 % of the analysed samples. In none of these specimens DMAE was detected. The presence of 4-CPA was confirmed in a selection of those samples according to WADA regulations (data not shown).

After oral administration of 200 mg of meclofenoxate hydrochloride, analyses of post administration urine samples provided the identification of DMAE and 4-CPA while meclofenoxate was not detected.

4. Discussion

As described in literature meclofenoxate is rapidly hydrolysed to DMAE and 4-CPA under physiological conditions. This was confirmed with the present study enabling only the detection of degradation products in post administration samples of meclofenoxate. The presence of 4-CPA in human urine is originating possibly from an oral intake of herbicide residues with food. The substance is utilized as a herbicide and growth regulator for plants. 4-CPA is considered to be absorbed completely from an oral dose and is eliminated rapidly unchanged in the urine [5]. The second degradation product DMAE as a nootropic substance is produced naturally in the human brain and is available as a nutritional supplement [6].

Frequent detection of 4-CPA in urine and the fact that DMAE occurs physiologically in the human body demonstrates the difficulty of sufficient screening for meclofenoxate. Hence an unambiguous proof of meclofenoxate misuse by identification of its degradation products requires further investigation.

References

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