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ORIGINAL ARTICLE

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Comparison of HPLC and CE for the analysis of dichlorprop in a case of intoxication

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Abstract A 49-year-old white male was found lying unconscious at home. He had vomited, his mouth was filled with a white foam and a pungent odour filled the room. After emergency treatment blood, urine and stomach contents were screened for drugs after acid and alkaline extraction with subsequent derivatisation and GC-MS analysis. Large quantites of 2,4-dichlorophenoxypropionic acid (dichlorprop, 2,4-DP) were found in all acid extracts. The man died 3 h later in hospital. Body fluids and tissues obtained at autopsy were analysed for 2,4-DP by high-performance liquid chromatography and capillary electrophoresis. The concentrations of 2,4-DP in cardiac blood, stomach contents, bile, liver, spleen, kidney and brain found by both methods were very similar.

Key words Dichlorprop · 2,4-DP · HPLC · Capillary electrophoresis

Introduction

2-(2,4-Dichlorophenoxy)propionic acid (2,4-DP, dichlorprop) was introduced in 1961 (Boots & Co.) as a selective herbicide. It belongs to the phenoxy acid group of herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D), which are pre- and post-emergence herbicides. The toxicity of these herbicides is relatively low; the LD-50 of 2,4-DP for rats is 825–1470 mg/kg after oral administration, > 4000 mg/kg after dermal administration and the LC-50 by inhalation administration is > 7.4 mg/l [1]. The analytical techniques used for measuring herbicides of the phenoxy acid group are high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and capillary electrophoresis (CE) [2]. Until now, no data have been published that demonstrate comparibility and reproducibility of quantification of 2,4-DP by CE.

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Case history

A 49-year-old man left home at 06.45 a.m. to his work in a garden centre. He was sent home 1 h later as he had obviously been under the influence of alcohol. When he arrived home his wife left and went to work. When she arrived home at 4 p.m., she found her husband in an unconscious state. He had vomited, his mouth was filled with a white foam and a pungent odour filled the room. He was admitted to hospital, where he arrived at 5 p.m. The man showed miosis, increased lacrimation, tachycardia (140/min), perspiration and a white foam in his mouth which in combination with the air exhaled gave a pungent odour.

With the suspicion of an intoxication, the stomach content was flushed and activated charcoal was given. Samples of the stomach contents, whole blood taken from the cubital vein and urine were obtained for toxicological analysis. The man became increasingly cyanotic and arrythmic and died the same day at 8.42 p.m.

The autopsy was performed 36 h after death. At autopsy a smell of solvent was obvious when the body cavities were opened. Additional findings were lung oedema, brain oedema, pronounced erosion of the stomach lining and a fatty liver. The following histological investigations showed massive hemostasis of the lungs in all capillaries and severe alveolar edema. Signs of disseminated muscle cell necrosis were found in myocardial fibers, focal with reactive cellular infiltration, indicating a prolonged period of agony. Furthermore interstitial edema of the fibers with erythrocyte diapedesis and acute osmotic nephrosis could be observed.

Cardiac blood, bile, stomach content and samples of liver, kidney, spleen and brain were collected for toxicological analysis. The post mortem and the hospital samples were stored at –20°C until analysis. The alcohol concentration of the blood taken post mortem from the femoral vein was 1.07‰. Later investigations by the police revealed that a bottle of Certrol DP was found in the house.

Material and methods

A comprehensive screening was performed on the samples taken at hospital (stomach contents, urine and whole blood from the cubital vein) by extraction first under acid conditions and secondly after alkalisation twice with diethylether. The extracts were evaporated to dryness. The acid extract was methylated with trimethyl-phenyl ammonia-hydroxide and the alkaline extract was acetylated with pyridine/acetic acid anhydride. The derivates were analysed by GC-MS.

2,4-Dichlorophenoxy-propionic acid methyl ester was detected in all of the acid extracts, which were methylated but Ioxynil, the minor component of Certrol DP could not be detected. In the further investigations, 2,4-dichlorophenoxy-propionic acid was the main substance found.

Quantitation of 2,4-DP by HPLC and CE

A stock solution of 2,4-DP was prepared in methanol (1 mg/ml). Drug-free whole blood was spiked with 0, 10, 50, 100, 250 und 500 µg/g of 2,4-DP. As an internal standard 2,4-dichlorophenoxy-acetic acid (2,4-D) was used at a concentration of 200 µg/g.

HPLC

Aliquots of 1 g of the spiked samples and the body fluids were mixed with 4 ml 1 M acetic acid, centrifuged and the supernatent was used for solid-phase extraction (C_{18}-column, 3-ml capacity, J.T. Baker). The elution of 2,4-DP was carried out with 3×500 µl acetone. The acetone extract was evaporated to dryness, redissolved in 1 ml of acetonitrile and injected into the HPLC-system.

The tissue samples from liver, kidney, spleen and brain were homogenized followed by enzyme digestion and 5 g of each sample was added to 25 ml 0.1 M tris buffer (tris-(hydroxymethyl)-aminomethane, pH10) and homogenized in an Ultra-Turrax with 500 mg of protease (Carlsberg Subtilisin, Boehringer). The samples were allowed to hydrolyse for 2 h in a water bath at 55° C. The homogenised solutions were transferred into 50 ml vials, with 5 ml glacial acetic acid and made up to 50 ml with aqua dest. (pH 4). After centrifugation 3 ml of this solution was used for the solid-phase extraction (SPE) [3].

HPLC was performed using a reversed-phase isocratic method with a Hewlett-Packard 1090 HPLC system and a Hewlett-Packard 1050 variable wavelength detector. A RP-select B column (250×4 mm; 5 µm particle size; Merck) was used. The mobile phase was a 5 mM monobasic potassium phosphate buffer (adjusted to pH 2.3 with o-phosphoric acid) – acetonitrile (55:45, v/v) with detection at 285 nm. The analysis was performed at 40° C and at a flow rate of 1.0 ml/min.

To study linearity and reproducibility, spiked whole blood samples were prepared, extracted and analysed in the same way as the samples of body fluids. All calculations were performed from chromatographic peak areas. The recovery was obtained from the same experiments, as peak area ratio between each extract and a respective non-extracted solution of 2,4-DP, corresponding to 100% extraction.

CE

The determination of 2,4-DP was carried out in the same spiked whole blood as used for HPLC. A very simple sample extraction was performed by mixing 200 µg of whole blood with 800 µl acetonitrile (ACN). The samples were centrifuged, the supernatant was transferred to a new vial, evaporated to dryness and redissolved in 400 µl demineralized water. This solution was used directly for the capillary electrophoretic determination.

Tissue samples were prepared in a similar way except that solid phase extraction was not necessary. After making up to 50 ml and centrifugation, 1 ml of the solution was extracted with 3 ml of diethylether. The upper phase was separated, evaporated to dryness and dissolved in 200 µl of water and acetonitrill (1:1, v/v) and was used for capillary electrophoresis. All separations were carried out on a P/ACE 5510 capillary electrophoresis system (Beckman) with a diode array detector (DAD).

The capillary zone electrophoresis was performed in untreated fused silica capillaries with a total length of 47 cm (40 cm effective length to the detector) and an internal diameter of 50 µm. Capillaries were treated before use by rinsing for 20 min with 0.1 M NaOH and after each sample with 0.1 M NaOH and running buffer for 1 min. Separations were carried out using a 50 mM monobasic potassium phosphate running buffer (pH 6,4), an applied voltage of 25 kV and the temperature was maintained at 20° C . Sample introduction was performed by hydrodynamic injection for 5 s. Electropherograms were monitored at 200 nm and the corrected peak areas were calculated using the Beckman System Gold software 8.10.

Results and discussion

In most cases of intoxication with herbicides of the phenoxyacid type, 2,4-dichlorophenoxy acetic acid was found as the toxic substance [4–7], but intoxications with 2,4-DP are rare. In the case presented, 2,4-DP could be identified as the methylester by GC-MS. For GC-MS pre-analytic derivatisation is frequently required, but it is widely regarded as the most specific analytical method available for drug detection and quantification. By this method only 2,4-DP could be detected in the body fluids.

For the quantification of the 2,4-DP concentrations in the different body fluids and tissue samples we applied the capillary electrophoresis (CE), a new technique which is becoming more common because of its non-chromatographic separation technique and easy sample preparation. This was compared with high-performance liquid chromatography which is typically applied for the analysis of herbicides.

Using HPLC the chromatographic retention time of 2,4-DP was 8.05 min, and that of the internal standard 2,4-D was 6.25 min. Regression on 12 points (six concentrations 0–500 µg/ml, two determinations per concentration) was good (r = 0.9999) and the recovery from whole blood ranged between 74–81%.

The migration times in the capillary electrophoretic separation were 4.80 min for 2,4-DP and 5.20 min for 2,4-D. The regression on 12 points (six concentrations 0–500 µg/ml, two determinations per concentration) was also good (r = 0.9999), and the recovery from whole blood ranged between 75–80%.

The solid phase extraction for the HPLC determination gave pure extracts with no interfering matrix peaks. Although the sample preparation needed for CE is much simpler, the results where qualitatively and quantitatively comparable due to the high resolution potential of this technique (Fig. 1).The application of the simple CE-sam-

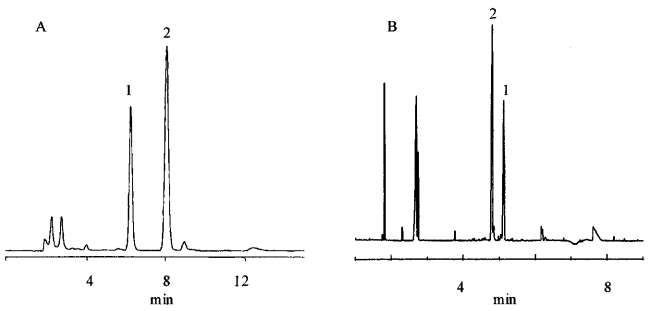

Fig. 1 The HPLC-chromatogram (A) and the CE-electropherogram (B) from the cardiacblood 1 = 2,4-D (ISTD), 2 = 2,4-DP

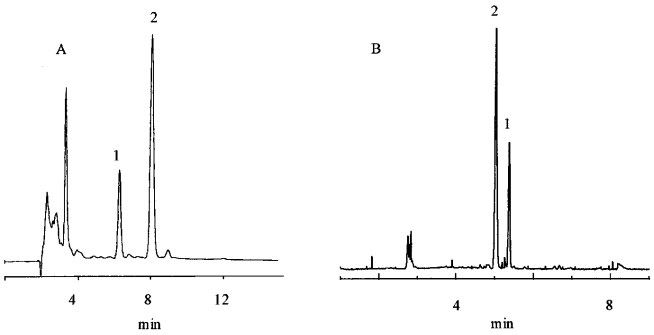

Fig. 2 The HPLC-chromatogram (A) and the CE-electropherogram (B) from the liver 1 = 2,4-D (ISTD), 2 = 2,4-DP

Table 1 Concentrations of 2,4-DP in body fluids taken after emergency treatment

Sample	2,4-DP concentration [µg/g] HPLC
Whole blood (cubital vein)	250
Stomach contents-flush	202
Urine	65

Table 2 Postmortem 2,4-DP concentrations in body fluids and tissues

Sample	2,4-DP concentrations [µg/g]	
	HPLC	CE
Whole blood (cardiac)	350	348
Stomach contents	n.d.	n.d.
Bile	372	386
Spleen	430	402
Kidney	413	397
Liver	619	605
Brain	113	116

n.d. not detected

ple preparation (200 µl cardiac blood + 800 µl ACN, mixing and centrifugation) results in extracts with a variety of interfering peaks. Also some of the matrix peaks showed a very long retention time, so that a column-wash procedure with a higher amount of ACN was necessary. The subsequent column recalibration was much more time-consuming than the SPE, although the life-span of the column increases with the purity of the applied extracts.

By CE the direct determination of 2,4-DP in tissues after enzymatic protein digestion without further extraction is possible, but not reproducible. The solution was very rich in polypetides, which stuck to the inner capillary wall. The migration times showed a wide variation, so that long rinsing periods were necessary to obtain reproducible migration times. A simple one-step extraction procedure with ether gave cleaner extracts, which could be analysed in a much shorter time. This procedure was less time consuming and therefore chosen (Fig. 2). All other fluids and organ extractions gave very similar results as the examples shown (Figs. 1, 2).

The signal-to-noise ratio achieved for the CE samples was sufficient for the determination of 2,4-DP in this case, but will be better in the cleaner extracts used for HPLC after SPE.

Because of their small sizes, the samples obtained from the emergency hospital were only analyzed by HPLC (Table 1), but the concentrations in the post mortem samples were measured with both methods and the results obtained were very similiar (Table 2).

Although the stomach wash had obiously removed all the herbicide (Table 2), an increase had occurred in the blood samples from 250 to 350 µg/g (Tables 1 and 2).

There are three possible explanations for this increase:

1. The whole blood samples in the emergency hospital were taken from the cubital vein, the post mortem whole blood sample was cardiac blood.
2. Some of the herbicide had already been transferred into the intestine and was not removed by the stomach flush.
3. Postmortem redistribution increased the blood level during the time between death and autopsy (36 h). This would neccessitate tissues with a great storage capacity for 2,4-DP which is unknown to us.

In conclusion, the analysis of 2,4-DP by capillary electrophoresis resulted in specific and quantifiable electropherogramms, comparable to the results obtained by HPLC. Since CE is associated with shorter times of analysis and a much simpler sample preparation, this methodology could become an attractive alternative to HPLC.

References

1. Industrieverband Agrar e.V. (1990) Wirkstoffe in Pflanzenschutz- und Schädlingsbekämpfungsmitteln, Physikalisch-chemische Daten und toxikologische Daten, 2. Aufl. BLV-Verlagsgesellschaft, München, pp 143–145
2. Garrison AW, Schmitt P, Kettrup A (1994) Separation of phenoxy acid herbicides and their enantiomeres by high-performance capillary electrophoresis. J Chromatogr 688 : 317–327
3. Scheurer J, Moore ChM (1992) Solid-phase extraction of drugs from biological tissues – a review. J Anal Toxicol 16 : 264–269
4. Coutselinis A, Kentarchou R, Boukis D (1977) Concentration levels of 2,4-D, and 2,4,5-T in forensic material. Forensic Sci 10 : 203–204
5. Osterloh J, Lotti M, Pond SM (1983) Toxicologic studies in a fatal overdose of 2,4-D, MCPP, and Chlopyrifos. J Anal Toxicol 7 : 125–129
6. Keller Th, Skopp G, Wu M, Aderjan R (1994) Fatal overdose of 2,4-dichlorophenoxyacetic acid (2,4-D). Forensic Sci 65 : 13–18
7. Kaa E, Gregersen M (1992) Fatal poisonings in Jutland (Danmark) during the 1980's. Int J Legal Med 105 : 133–138