Acute poisoning with a rat bait

LC-ESI-MS for simultaneous determination of vitamin K antagonists in h

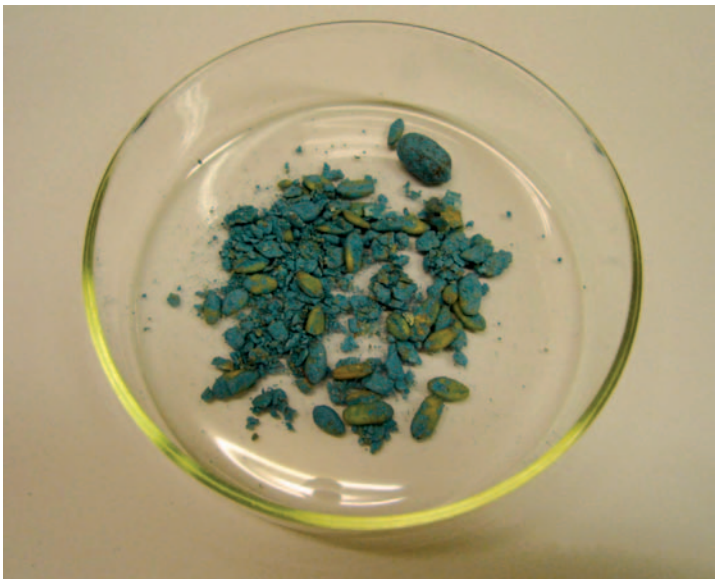

Figure 1: Bait preparation ingested by the patient

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Solving acute poisoning cases, as well as therapeutic drug monitoring and drug testing are among the major tasks of the Clinical Toxicology Department of the BBGes. This example describes an LC-MS method for the determination of rat poison intoxications.

A 62-year old man was admitted to the emergency station with symptoms of nausea and vomiting. He admitted to having ingested rat poison bait in a suicide attempt. The patient's medical history indicated an earlier suicide attempt in 2003. No psychiatric treatment had followed. A blood sample of the patient together with a sample of blue rat poison bait (Figure 1) was sent to the BBGes Toxicology Department's laboratory. Analysis using LC-ESI-MS confirmed that poisoning was due to the rat poison's active compound coumatetralyl.

Vitamin K antagonists

4-Hydroxycoumarin, a derivative of the sweet woodruff phytochemical coumarin, and 1.3-indandione belong to a group of indirect anticoagulants (Figure 2). They are also known as vitamin K antagonists as their action is based on a functional disruption of the synthesis of vitamin K-dependent coagulation factors.

Based on the half-life values of the coagulation factors already circulating in the bloodstream, the anti-coagulating effects of the rat poison are evident only after a certain time delay.

Some vitamin K antagonists (acenocoumarol, phenprocoumon, warfarin) are used therapeutically as anticoagulants. The same anticoagulant effects are also being used effectively to combat rodents. Due to increasing resistance of various rat species to these compounds, second-generation anticoagulants – the so-called superwarfarins – have been developed. These are highly effective, even at very low dosages and they have long half-lives associated with long pharmacological action. With increasing commercial distribution of superwarfarins, the number of poisoning cases in humans and animals has also increased. In 2004 the poisoning emergency center in Berlin alone already registered 110 poisoning cases involving anticoagulant ingestion.

Depending on the formulation, the active ingredient concentrations of rat poison baits permitted in Germany are between 0.0025 and 0.79 %. Combination preparations, for instance Racumin® Plus oat flake bait (coumatetralyl and cholecalciferol, Bayer CropScience) or Celaflor® Brumolin® (difethialon and sulfachinoxalin, Scotts Celaflor GmbH & Co.) are also available.

Poisoning cases in humans and animals are indicated by an increased tendency towards bleeding. Death occurs via internal bleeding or haemorrhagic shock. The active compound coumatetralyl has therefore been rated as extremely toxic (T+) in the German Toxic Substance Act. The toxicity data for coumatetralyl and coumatetralyl-containing rat poison baits in various forms of application are summarized in Table 1.

The most important diagnostic parameters are the blood coagulation values (INR [International Normalized Ratio] and the Quick-value). Immediate measures in poisoning cases involving anticoagulants are decontamination and, where necessary, administration of an antidote (vitamin K1). When bleeding is acute and life threatening, the decreased level of coagulation factors must first be replenished.

LC-MS analysis

For the detection and quantification of coumatetralyl, an LC-MS method was used that had been developed specifically for toxicology analyses at the BBGes. This procedure is suitable for simultaneous identification and quantification of 10 indirect anticoagulants in human serum. The method is based on an acidic (pH = 4.2) liquid-liquid extraction using 1-chlorobutane with subsequent detection via LC-ESI-MS. This simple method for the determination of vitamin K antagonists offers broad compound screening and high selectivities with short

Name	LD_{50} Rat (m) oral (mg/kg KG)	LD_{50} Rat (m) transdermal (mg/kg KG)	LD_{50} Rat (m) via inhalation (mg/L)
Coumatetralyl	30.15 (f)	> 100 < 500 (f)	0.05 (dust)
Racumin powder	5000	> 5000	> 3.3
Racumin baits	> 5000	> 5000	– –

Table 1: Toxicity data for coumatetralyl: m = male, f = female

(From "Wirkstoffe in Pflanzenschutz und Schädlingsbekämpfungsmitteln: physikalisch-chemische und toxikologische Daten", 3. Aufl., Industrieverband Agrar e.V. BLV, München, 2000)

uman serum

analysis times using two masses for identification/quantification. Figure 3 shows the compounds identified using this method.

Based on suspected poisoning with a coumarin derivative, serum samples as well as bait samples were subjected to liquid-liquid extraction and subsequently analyzed according to the LC-MS method described below. In addition, a systematic toxicological analysis (STA) was carried out using HPLC-DAD. The bait samples were first extracted using methanol, the resulting solution was then filtered after centrifugation and 5 µL was injected directly into the LC-MS system (for LC-MS conditions see insert).

For quantification, a 6-point calibration was carried out. The calibration was linear for all calibration points in the range from 10 up to 250 µg/L ($r^2 > 0.995$). The determination limit was 5 µg/L (S/N 10). For the precision the following coefficients of variation were determined based on quality control samples (day-to-day, n = 6, 100 µg/L): acenocoumarol (5.2 %), coumachlor (5.9 %), coumatetralyl (4.8 %), phenprocoumon (6.0 %), warfarin (6.0 %), brodifacoum (14.9 %), bromadiolon (8.2 %), difenacoum (8.4 %), difethialon (11.7 %), flocoumafen (14.2 %).

Results

The selected analytical conditions enable fast elution of the listed compounds within 5 min at a determination limit of 10 µg/L. The experimental conditions (mobile phase, analytical column, ESI interface) conform to the usual LC-MS system configurations at the institute laboratory

LC-MS conditions

HPLC

Binary pump system with membrane degasser, autosampler, oven, UV detector (Shimadzu)

Mobile phase: A: methanol, B: methanol/0.1 % HCOOH (10/90, v/v) · **Flowrate:** 0.60 mL/min · **Gradient:** 0 – 0.7 min: 95 % B; 0.7 – 1.1 min: 50 % B linear; 1.1 – 3.2 min: 6 % B linear; 3.2 – 3.8 min: 6 % B; 3.8 – 4.2 min: 95 % linear · **Separation column:** Atlantis C18 (2.1 x 20 mm, 3 µm, Waters) · **Oven temperature:** 40 °C · **Gradient for the determination of cholecalciferol (vitamin D3):** 0 – 0.7 min: 20 % B; 0.7 – 3.5 min: 6 % B linear; 3.5 – 3.7 min: 6 % B; 3.7 – 4.1 min: 20 % B linear; 4.1 – 4.2 min: 20 %.

MS

LCMS-2010 system (Shimadzu)

Ion source: ESI positive & negative · **Nebulizer gas:** nitrogen; 4,5 L/min · **Block- and CDL temperature:** 300 °C · **Detector voltage:** 1.9 kV **SIM-masses:** acenocoumarol ESI (-): 353 & 352; brodifacoum ESI (-): 523 & 521; bromadiolon ESI (-): 527 & 525; coumachlor ESI (-): 343 & 341; coumatetralyl ESI (-): 292 & 291; difenacoum ESI (-): 445 & 443; difethialon ESI (-) 539 & 537; flocoumafen ESI (-) 543 & 542; phenprocoumon ESI (-): 280 & 279; warfarin ESI (-) 308 & 307, internal standard ESI (+): 432

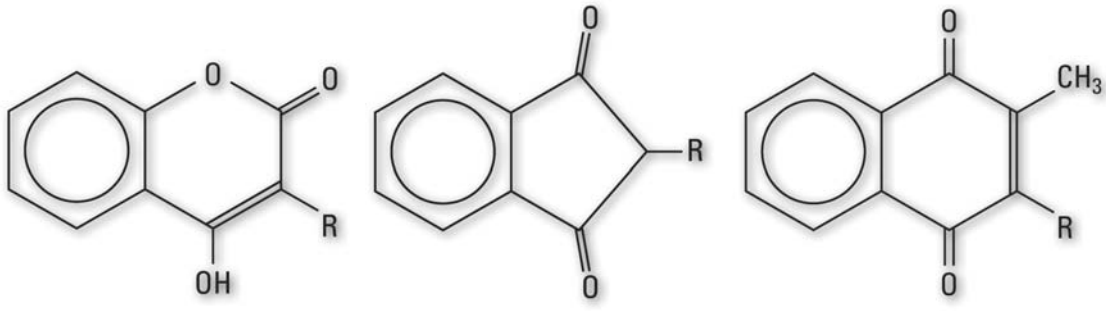

Figure 2: Structural similarity between vitamin K and its antagonists

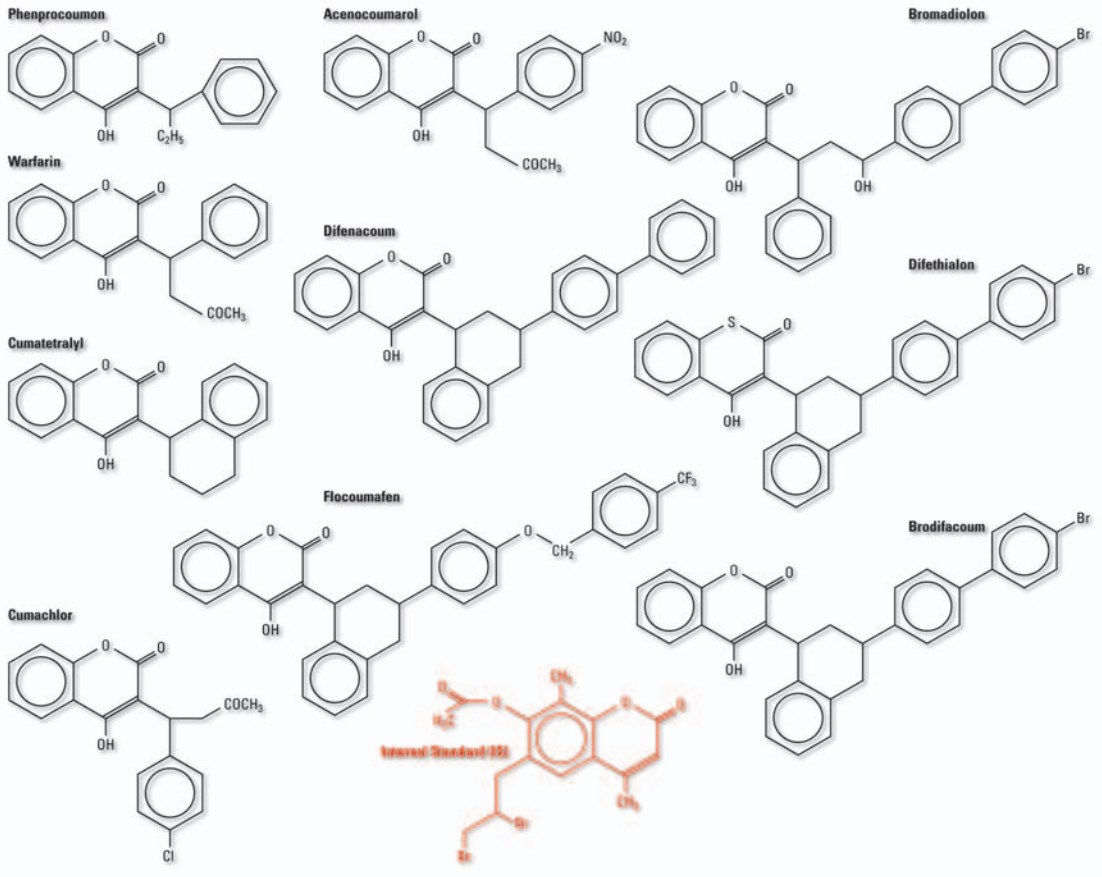

Figure 3: Structures of the vitamin K antagonists and the internal standard

and therefore do not require long reconfiguration times.

Figure 4 shows SIM chromatograms of the 10 established vitamin K antagonists (standard each 100 µg/L). The symmetry of the peaks is acceptable. To safeguard the validity of the analytical result, two masses were used. In marked contrast with the identification after fragmentation in a first run and quantification after a second run, our analytical strategy enabled a simultaneous identification and quantification within one run due to the high sensitivity, whereby a higher sensitivity was obtained for mass [M-1 amu]. Within the scope of method validation, 12 tested blank samples obtained via 6 different sampling methods were free from the above-mentioned vitamin K antagonists.

The upper determination limit of 250 µg/L (3 decades) is not sufficient for the determination of phenprocoumon and warfarin (therapeutic range up to 3000 µg/L). Therefore for the investigation of these compounds, correspondingly less material should be used or the sample should be diluted with coumarin-free blank plasma/serum. The precision of the method is suitable for obtaining acceptable results at low analysis frequency when time is essential.

Based on the suspected coumarin intoxication, the patient sample was investigated using the above-described analytical procedure.

The active compound could be identified unequivocally from the chromatogram (Figure 5) via the retention time (2.0 min) as well as the two masses (m/z 292 and 291). Quantification resulted in a coumarin concentration of 121 µg/L. The control sample (expected value 100 µg/L) showed a coumatetralyl concentration of 94.7 µg/L which was within the required range of 80 – 120 µg/L (± 20 % of the expected value). In addition, the sample underwent screening for basic compounds (HPLC-DAD) whereby no further compounds were identified.

In the bait sample the active compound coumatetralyl was also identified via the retention time and the two characteristic masses. There were no interfering peaks. Both results pointed to a coumatetralyl-containing rodenticide bait. In order to test whether the rodenticide sample was the combination preparation Racumin® Plus oat flake bait (Bayer AG), a methanol extract of the rodenticide sample was analyzed via LC-APCI-MS to detect cholecalciferol. However, no indication on the presence of cholecalciferol was found.

Although a coumatetralyl concentration of 121 µg/L was measured when the patient was admitted, coumatetralyl could no longer be detected in a control sample taken eleven days later (LOQ = 10 µg/L). This is due either to a distinctive distribution behavior or a half-life of less than two to three days.

Summary

This example of a suicide attempt with a coumatetralyl-containing rat poison was presented to demonstrate a sensitive and reliable analytical method for the simultaneous determination of 10 vitamin K antagonists (among them 5 superwarfarins) in human serum/plasma using LC-ESI-MS. The method is based on a simple and fast liquid-liquid extraction with subsequent LC-MS analysis.

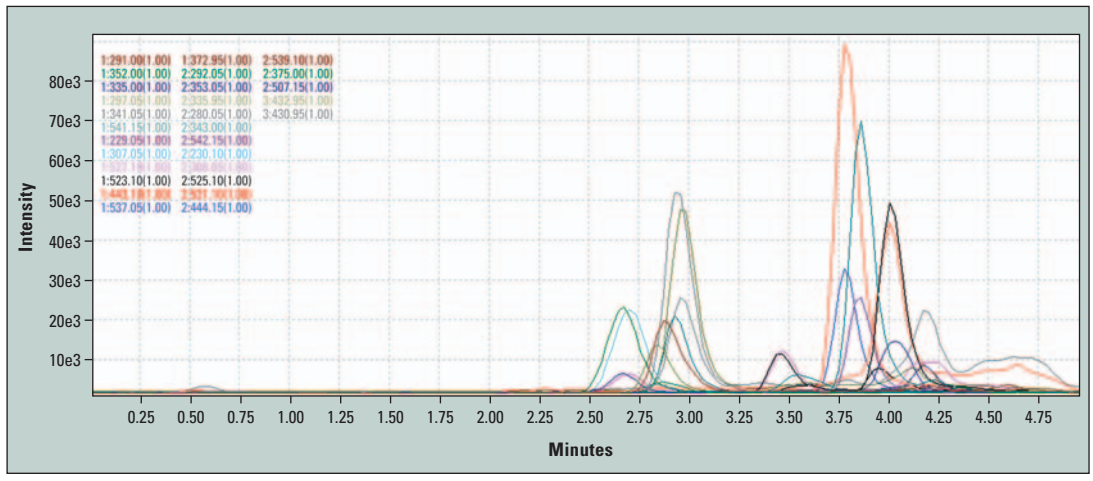

Figure 4: SIM chromatograms of the 10 vitamin K antagonists (each 100 µg/L, ESI negative)

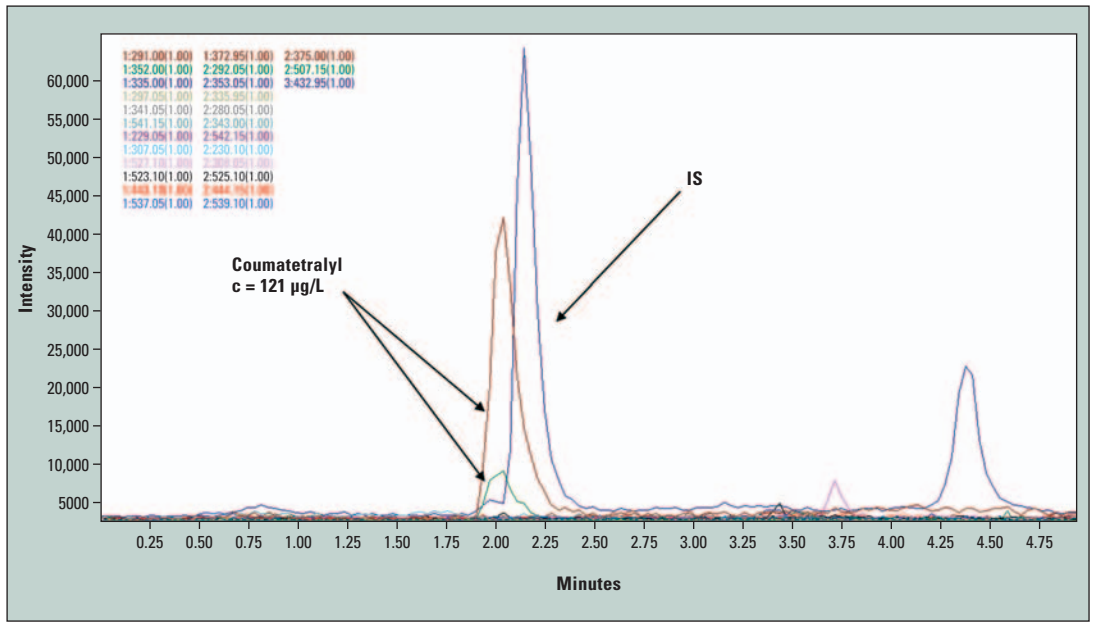

Figure 5: Serum sample of the patient with internal standard (SIM chromatogram)