REPORT TITLE

Dodine: Development and Validation of a Confirmatory Method for the Determination of Dodine in Surface Water

OCSPP Guideline
850.6100

STUDY COMPLETION DATE

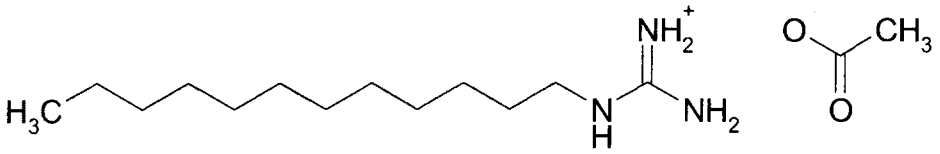
21-Sep-07

1. INTRODUCTION

Background and Objective:

The objective of the study was to develop and to validate a confirmatory method for the determination of dodine (dodecylguanidine) in surface water, with a target limit of quantitation (LOQ) of 0.05 µg/L.

Dodine



Method Principles:

100 mL of water (V_{Ex}) was mixed with 10 mL of phosphate buffer (pH 7). Subsequently the mixture was transferred onto a Waters Oasis HLB SPE cartridge. The water specimen was sucked through the SPE cartridge. Then the column was first rinsed with two different washing solutions and finally eluted with 10 mL of a mixture of acetonitrile/methanol/2% acetic acid, 49/21/30 v/v/v. The final extract was analysed by LC/MS/MS, monitoring two daughter ions for quantitative evaluation.

Method Validation:

The analytical method was validated by analysing for surface water 2 blank control specimens, 5 replicate specimens fortified at LOQ (0.05 µg/L), and 5 replicate specimens fortified at 10xLOQ (0.50 µg/L).

2. EXPERIMENTAL

2.1 Test System

Surface (river) water collected from the Danube River on 16-Apr-07 near the Bundesstrasse B30 in Ulm (Germany). The water was yellow/brownish, musty in smell, pH was 7.6, total hardness was 2.4 mmol/L corresponding to 13.2 °dH. The surface water was characterized by accredited Institute Alpha (Ulm, Germany, following common DIN or EN guidelines and methods), with the following results (non-GLP):

DOC (dissolved organic carbon, EN 1484:1997): 3.8 mg/L.
Silt content (filtered particles, DIN 38 409 H 2-3): 464 mg/L.
Turbidity, nephelometrically (EN 7027:1999):
(undissolved particles homogenized prior to measurement): 87 NTU

2.2 Test / Reference Item

A standard of dodine was provided by the Sponsor with a Certificate of Analysis (see Appendix 1) and used as test/reference item.

Dodine

IUPAC Name:	1-Dodecylguanidinium acetate
CAS No.:	Acetate: [*2439-10-3*].
	Free base: [*112-65-2*]
Chemical Formula:	$C_{15}H_{33}N_3O_2$
	Free base: $C_{13}H_{29}N_3$
Molecular Mass:	Acetate: 287.4 g/mol.
	Free base: 227.4 g/mol.
Purity:	95.1 %.
Expiration Date:	July 2008.

2.3 Equipment and Instrumentation

2.3.1 Equipment for Extraction and Specimen Clean-up

Sartorius balance RC 210D for analytical standards, laboratory balance Scaltec SBC 41.
Ultrasonic bath Elma Transsonic 700.
Baker SPE-100 SPE station. Denver Instruments pH meter.
Common laboratory equipment, common laboratory glass ware (cleaned in a laboratory dishwasher and air-dried before use).

2.3.2 LC/MS/MS Instrumentation

Agilent 1200 Series HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.

Applied Biosystems MDS Sciex API 4000 triple quadrupole LC/MS/MS system with TurboIonspray ESI source. Analyst 1.4.1 Instrument control and data acquisition software.

Thermo Aquasil C_{18} column (length: 150 mm, i.d.: 3.0 mm, particle size: 3 µm).
Pre-column: Phenomenex C_{18} 4 mm x 3 mm.

2.4 Reagents, Chemicals and Miscellaneous

Solvent/Chemical/Supply	Grade	Supplier
Acetonitrile	HPLC Grade	Promochem
Methanol	HPLC Grade	Promochem
Aqua Ampuwa	Bidistilled	Fresenius
2-Propanol	99.5 %	Promochem
Ammonia	25 %	Merck
Formic acid	98 100 %	Riedel de Haën
Glacial acetic acid	100 %	Merck
Potassium dihydrogenphosphate	99.5 %	Fluka
Sodium hydroxide	99 %	Merck
Waters Oasis HLB SPE cartridges	0.2 g, 6 mL	Waters

The following reagent solutions were prepared for specimen preparation.

0.05 M Phosphate Buffer Solution:

3.40 g of potassium dihydrogenphosphate was dissolved in 250 mL of bidistilled water. 1 M sodium hydroxide solution was added to adjust a pH of 7. Finally the volume was adjusted to 500 mL using water.

Wash Solution 1:

21 mL of acetonitrile and 9 mL of methanol were mixed with 70 mL of an aqueous 0.5 % ammonia solution.

Wash Solution 2:

21 mL of acetonitrile and 9 mL of methanol were mixed with 70 mL of an aqueous 2 % acetic acid solution.

Elution Solution:

49 mL of acetonitrile and 21 mL of methanol were mixed with 30 mL of an aqueous 2 % acetic acid solution.

2.5 Standard Solutions

See Appendix 1 for information provided with the analytical test/reference item used.

2.5.1 Stock Solution

A 1.0 mg/mL stock solution of the analyte was prepared in 2-propanol by accurately weighing 10.41 mg of dodine (purity 95.1 %) into a 10-mL volumetric flask, using an analytical balance.

2.5.2 Fortification Solutions

The following fortification solutions of the analyte were prepared in methanol:

Use solution with	Pipette [µL]	Dilute to [mL]	Obtain [µg/mL]
1.0 mg/mL (stock solution)	1000	20	50
50 µg/mL	2000	20	0.50
5.0 µg/mL	2000	20	0.50

2.5.3 Calibration Solutions in Solvent

The stock standard solution was used to prepare by volumetric dilution into methanol an intermediate solution containing dodine at a concentration of 10 µg/mL. This intermediate solution was used for further volumetrical dilutions into methanol to obtain the following calibration solutions (examples, may be modified):

1.0 µg/mL, 0.10 µg/mL, 0.050 µg/mL, 0.020 µg/mL.

The calibration solutions in solvent were not directly used for LC/MS/MS analysis, but partly used for preparation of matrix-matched standards.

2.5.4 Matrix-Matched Calibration Solutions

Matrix-matched calibration solutions were prepared for evaluation of surface water specimens. The analytical method was assessed and subsequently validated according to SANCO requirements using LC/MS/MS determination.

Matrix-matched calibration solutions were prepared by performing extraction and clean-up with residue-free control specimens. For the preparation of matrix-matched standards aliquots of the raw extracts (V_{Ex} = 100 mL) of these specimens were fortified with dodine using calibration solutions in solvent as follows (examples):

Use solution with [ng/mL]	Pipette [mL]	Add matrix extract [mL]	Obtain [ng/mL]
1000	0.020	0.980	20
1000	0.010	0.990	10
100	0.050	0.950	5.0
20	0.050	0.950	1.0
50	0.010	0.990	0.50
20	0.005	0.995	0.10

These solutions were used for external calibration of specimen extracts.

2.5.5 Storage and Stability of Standard Solutions

All standard solutions were stored refrigerated when not in use. Stability was not tested specifically, however, the solutions are considered stable for the duration of the analytical phase of the study when kept at < 8 °C, as demonstrated by consistent LC/MS/MS results.

3. ANALYTICAL PROCEDURE

The analytical method for the determination of dodine in water was initially developed and validated at NOTOX[1]. The analytical method was adapted and subsequently validated according to SANCO requirements using LC/MS/MS determination.

3.1 Preparation of Specimens and Fortification

10 mL of phosphate buffer solution was added to a water specimen (V_{Water} = 100 mL) placed in a measuring flask.

Recovery control specimens were fortified with 0.10 mL of either the fortification solution with 0.050 µg/mL (LOQ) or 0.50 µg/mL (10xLOQ), to obtain dodine fortification levels of 0.05 µg/L or 0.50 µg/L.

3.2 Solid Phase Extraction and Preparation of Final Extract

1. A Waters Oasis HLB cartridge (0.2 g, 6 mL) was conditioned with 1 mL of methanol, followed by 1 mL of purified water.
2. The water specimen was transferred onto the SPE cartridge and sucked through the cartridge.
3. The measuring flask was rinsed with 3 mL of purified water. The rinse was transferred onto the SPE cartridge.
4. The SPE cartridge was washed with 2 mL of washing solution 1, followed by 2 mL of washing solution 2. The washing solutions were discarded.
5. Dodine was eluted from the SPE cartridge using 10 mL of the elution solution.[2]
6. The eluate was evaporated to approx. 3 mL using a rotary evaporator.
7. Adjust a volume of 5.0 mL (V_{End}) by addition of methanol/water (2/8 v/v).
8. Analyse final extract by LC/MS/MS.

3.3 LC/MS/MS Determination

3.3.1 RP-HPLC Method

The following RP-HPLC method was used:

HPLC System	Agilent 1200 HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.
HPLC Column	Thermo Aquasil C_{18} column: Length: 150 mm, i.d.: 3.0 mm, particle size: 3 µm. Pre-column: Phenomenex C_{18}, 4 mm x 3 mm. 20 °C column oven.
HPLC Injection Volume	20 µL.
HPLC Method	Solvent A: 1 % formic acid in water. Solvent B: 0.1 % formic acid in methanol Mobile Phase Composition: Time (min) / Flow rate (mL/min) / % A / % B 0.0 / 0.40 / 80 / 20 5.0 / 0.40 / 0 / 100 14.0 / 0.40 / 0 / 100 14.1 / 0.40 / 80 / 20 18.0 / 0.40 / 80 / 20
Retention Time	Dodine: approx. 8.9 min

3.3.2 MS/MS Detection Method

LC/MS/MS employed electron spray ionization and used the free base $[M+H]^+$ with 228 m/z as parent ion for MS/MS detection of dodine. Two daughter ions were monitored (187 m/z and 60 m/z).

The following LC/MS/MS method was used for determination of dodine:

MS System	Applied Biosystems MDS Sciex API 4000 triple quadrupole LC/MS/MS system with TurboIonspray (ESI) source
Ion Source Conditions ESI Positive Polarity	Source temperature: 550 °C Gas supply GS 1: 40 (arbitrary units) Gas supply GS 2: 70 (arbitrary units) Curtain gas: 20 (arbitrary units) Entrance potential: 10 V IonSpray voltage: 5000 V Declustering potential: 90 V
MS/MS Conditions	*Dodine (228 m/z → 187 m/z):* CE: 25 V CXP: 13.3 CAD: 5 (arbitrary units) *Dodine (228 m/z → 60 m/z for confirmation):* CE: 36.6 V CXP: 4.0 V CAD: 5 (arbitrary units)
MS/MS Method	*Dodine:* Parent ion: m/z 228.3 Daughter ions: m/z 187, used for quantitation. m/z 60, used for confirmation Dwell time per transition: 250 ms

3.3.3 Calibration and Evaluation Procedures

The quantitative determination was carried out by external standardization with calibration standards in matrix extracts.

3.4 Calculation of Results

3.4.1 Calculation

Calculation of results is based on peak area measurements. The concentrations of the analyte in the final extracts are calculated from the linear calibration functions obtained by linear regression calculation, using "1/X" weighting by the Analyst LC/MS/MS software.

The individual residues in the specimens [in µg/L] are calculated as shown in the following equation:

$$R = C_{End} \times (V_{End} / V_{Water}) / 1000 \text{ ng/µg}$$
$$= C_{End} \times \text{Multiplier M}$$

Where :

R: Analyte residue in µg/L.

C_{End}: Concentration of analyte in final extract, in ng/mL.
(where multiple injections were evaluated: average).

V_{Water}: Volume of water specimen: 0.10 L.

V_{End}: Volume of final extract: 5.0 mL.

1000: Divisor used to adjust for dimensions, converts ng to µg.

Recoveries (in %) are calculated as follows:

$$Rec. = (R / R_{fortified}) \times 100 \%.$$

3.4.2 Example for Calculation

PTRL specimen ID P1271-49 (see Table 1 for calculation and evaluation and Figure 6 for LC/MS/MS chromatograms) was fortified at 0.050 µg/L.

The LC/MS/MS injection was evaluated by the instrument software using the external calibration function shown in Figure 1. Using the MS/MS transition 228 m/z → 187 m/z, quantification resulted in a final concentration for dodine of 0.944 ng/mL.

The residue R for dodine was calculated as follows:

$$R = C_{End} \times (V_{End} / V_{Water}) / 1000 \text{ ng/µg}$$
$$= C_{End} \times \text{Multiplier M}$$

= 0.944 ng/mL x (5.0 mL) / (0.1 L) / 1000 ng/µg

= 0.944 x 0.050 µg/L

= 0.0472 µg/L

The recovery is calculated with:

Rec. = $(R / R_{fortified}) \times 100\ \%$

$(0.0472\ \mu g/L / 0.050\ \mu g/L) \times 100\ \%$ 94 %.

4. RESULTS AND DISCUSSION

The objective of the study was to develop and to validate a confirmatory method for the determination of dodine (dodecylguanidine) in surface water, with a target limit of quantitation (LOQ) of 0.05 µg/L.

Specimen preparation for surface water used solid-phase extraction (SPE). Determination was performed using LC/MS/MS, monitoring two daughter ions for quantitative evaluation.

The analytical method was validated successfully by analysing 2 blank control specimens, 5 replicate specimens fortified at LOQ (0.050 µg/L), and 5 replicate specimens fortified at 10xLOQ (0.50 µg/L).

5. CONCLUSIONS

An analytical method was successfully validated for the determination of dodine in surface water.

The method was shown to be highly selective, as it includes two parent-daughter ion transitions, and it yields accurate and repeatable results. The limit of quantification (LOQ) was established at 0.050 µg/L, the limit of detection (LOD) at 0.005 µg/L.

The method fulfills the requirements of the Council Directive 91/414/EEC Annex II (Part A, Section 4.2.), detailed in the EC Guidance document on residue analytical methods (SANCO/825/00 rev. 7 17/03/04). It is applicable for enforcement and monitoring purposes.