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Method for the determination of the metabolite residues of furaltadon, nitrofurazone, nitrofurantoin and furazolidone in honey - Liquid chromatography - tandem mass spectrometry method

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Foreword

Annex A and Annex B in this Part of GB/T 18932 are informative.

This Part was proposed by Qinhuangdao Entry-exit Inspection and Quarantine Bureau of the People's Republic of China.

The Part shall be under the jurisdiction of China Supply and Marketing Cooperatives.

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This Part is first-time released national standard.

Method for the determination of the metabolite residues of furaltadon, nitrofurazone, nitrofurantoin and furazolidone in honey - Liquid chromatography - tandem mass spectrometry method

1 Scope

This Part of GB/T 18932 specifies the liquid chromatography - tandem mass spectrometry determination method for the metabolite residues of 5-methylmorpholine-3-amino-2-yl alkyl ketones in furaltadon, semicarbazide in nitrofurazone, 1-amino-2-hydantoin in nitrofurantoin and 3-amino-2-yl alkyl ketones in furazolidone in honey.

This Part applies to the determination of the residues of 5-methylmorpholine-3-amino-2-yl alkyl ketones, semicarbazide, 1-amino-2-hydantoin and 3-amino-2-yl alkyl ketones in honey.

The detection limit of the method in this Part: 0.2μg/kg for 5-methylmorpholine-3-amino-2-yl alkyl ketones and 3-amino-2-yl alkyl ketones; 0.5μg/kg for semicarbazide and 1-amino-2-hydantoin.

2 Normative references

The provisions in following documents become the provisions of this Part through reference in this Part of GB/T 18932. For dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Part, however, parties who reach an agreement based on this Part are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6379 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory tests (GB/T 6379-1986, neq ISO 5725:1981)

GB/T 6682 Water for analytical laboratory use - Specification and test methods (GB/T 6682-1992, neq ISO 3696:1987)

3 Principle

column moist.

4.16 0.45μm filter membrane.

4.17 5-methylmorpholine-3-amino-2-yl alkyl ketones, semicarbazide, 1-amino-2-hydantoin and 3-amino-2-yl alkyl ketones reference material: Purity ≥99%.

4.18 5-methylmorpholin-3-amino-2-yl alkyl ketone, semicarbazide, 1-amino-2-hydantoin and 3-amino-2-yl alkyl ketones standard stock solution: 1.0mg/mL. WEIGH appropriate amount of the reference material of four kinds of nitrofuran metabolites (4.17); USE methanol to prepare 1.0mg/mL standard stock solution. The stock solution shall be stored in a freezer of -18°C, away from lights; the storage period is three months.

4.19 5-methylmorpholin-3-amino-2-yl alkyl ketone, semicarbazide, 1-amino-2-hydantoin and 3-amino-2-yl alkyl ketone mixed standard working solution: 1.0μg/mL. PIPETTE appropriate amount of the standard stock solution of four kinds of nitrofuran metabolites (4.18); USE methanol to prepare 1.0μg/mL mixed standard working solution. The standard working solution shall be stored in a freezer of -18°C, away from lights; the storage period is three months; RESUME to room temperature before use.

4.20 5-methylmorpholin-3-amino-2-yl alkyl ketone, semicarbazide, 1-amino-2-hydantoin and 3-amino-2-yl alkyl ketone mixed matrix standard calibration solution: WEIGH 7 honey blank samples (the sample weight is 4g) in 50mL brown centrifuge tubes; ADD appropriate amount of the mixed standard working solution of four kinds of nitrofuran metabolites (4.19) in 6 blank samples respectively; OPERATE synchronously in accordance with the sample operation procedures. So that the concentration of 5-methylmorpholin-3-amino-2-yl alkyl ketone and 3-amino-2-yl alkyl ketones are respectively 0ng/mL, 0.5ng/mL, 1.0ng/mL, 2.0ng/mL, 5.0ng/mL, 10.0ng/mL and 20.0ng/mL; the concentration of semicarbazide and 1-amino-2-hydantoin are respectively 0ng/mL, 1.0ng/mL, 2.0ng/mL, 5.0ng/mL, 10.0ng/mL, 20.0ng/mL and 50.0ng/mL in the final sample solutions; take them as matrix standard calibration solution. This matrix standard calibration solution is prepared when it is to be used.

5 Instruments

5.1 Liquid chromatography - tandem quadrupole mass spectrometer, equipped with electrospray ion source.

5.2 Analytical balances: 2 balances with sensitivity of 0.1mg and 0.01g respectively.

5.3 Liquid mixer.

5.4 Solid phase extraction device.

to wash solid phase extraction column; DISCARD all of the effluent. USE a vacuum pump (5.7) to drain the Oasis HLB solid phase extraction column for 10min at 65kPa negative pressure. USE 4mL of ethyl acetate (4.3) to elute the analyte; COLLECT the eluent in a 25mL brown centrifuge tube; DRY in a nitrogen blowing instrument (5.5) by 40°C water bath; USE 1mL of sample constant-volume solution (4.14) to dilute to constant volume. After the constant-volume solution is mixed, MAKE it pass through the 0.45μm filter membrane (4.16); the filtrate is used for determination by liquid chromatography - tandem mass spectrometry.

7.3 Determination

7.3.1 Liquid chromatography conditions

a) Chromatographic column: ZORBAX SB-C18, 3.5μm, 150mm × 2.1mm (diameter) or the equivalent;

b) Column temperature: 30°C;

c) Injection volume: 40μL;

d) Mobile phase and flow rate are shown in Table 1.

Table 1 Liquid chromatography gradient elution conditions

Time/min	Flow rate/(μL/min)	0.4% acetic acid aqueous solution/(%)	Acetonitrile/(%)
0.00	200	70	30
3.00	200	70	30
3.01	200	20	80
8.00	200	20	80
8.01	200	70	30
15.00	200	70	30

7.3.2 Mass spectrometer conditions

a) Ion source: electrospray ion source (ESI);

b) Scan mode: positive ion scan;

c) Detection method: multiple reaction monitoring (MRM);

d) Electrospray ionization voltage (IS): 5500V;

e) Nebulizer (NEB) pressure: 0.076MPa;

f) Curtain air (CUR) pressure: 0.069MPa;

g) Collision air (CAD) pressure: 0.030MPa;

(μg/kg);

c - Concentration of the tested component solution, obtained from standard working curve, in nanograms per milliliter (ng/mL);

V - Final constant volume of the sample solution, in milliliters (mL);

m - Mass of the final sample represented by sample solution, in grams (g).

Note: the blank values shall be deducted from the calculation results.

9 Precision

The precision data in this Part are determined in accordance with the specifications of GB/T 6379. The repeatability and reproducibility values are calculated by 95% confidence.

9.1 Repeatability

Under repeatability conditions, the absolute difference between two independent test results obtained shall not exceed the repeatability limit (*r*). The content range and repeatability equation of four kinds of nitrofuran metabolites in honey are shown in Table 4.

If the difference exceeds the repeatability limit, it shall abandon the test results and re-complete the determination of two single tests.

9.2 Reproducibility

Under reproducibility conditions, the absolute difference between two independent test results obtained shall not exceed reproducibility limit (*R*). The content range and reproducibility equation of four kinds of nitrofuran metabolites in honey are shown in Table 4.

Table 4 Content range AND repeatability and reproducibility equations

Name of nitrofuran metabolites	Content range/(μg/kg)	Repeatability limit *r*	Reproducibility *R*
5-methylmorpholine-3-amino-2-yl alkyl ketones	0.2~4	lgr=0.9656lgm - 1.0897	lgR=0.9625lgm - 0.7128
semicarbazide	0.5~10	lgr=0.9660lgm - 1.1445	lgR=0.9152lgm - 0.6513
1-amino-2-hydantoin	0.5~10	lgr=0.9844lgm - 1.0935	R=0.2058m + 0.0119
3-amino-2-yl alkyl	0.2~4	r=0.0735m + 0.0016	lgR=1.0813lgm - 0.7982

Annex B

(Informative)

Recovery rate

Test data of the added concentration and the average recovery rate of four kinds of nitrofuran metabolites in this method are shown in Table B.1.

Table B.1 Test data of the added concentration and the average recovery rate of four kinds of nitrofuran metabolites

Name of nitrofuran metabolites	Added concentration/ (μg/kg)	Recovery rate/ (%)
5-methylmorpholine-3-amino-2-yl alkyl ketones	0.2	96.7
	0.4	93.0
	2	90.9
	4	89.5
semicarbazide	0.5	96.4
	1.0	93.3
	5.0	88.7
	10	88.9
1-amino-2-hydantoin	0.5	97.5
	1.0	93.8
	5.0	91.9
	10	87.3
3-amino-2-yl alkyl ketones	0.2	92.9
	0.4	92.6
	2	89.6
	4	88.1

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