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RESEARCH ARTICLE

Single-Run method for Antibiotics in Honey through UPLC-MS/MS

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ABSTRACT

The current study focused on the development of a single method for quantitative estimation of the compounds of honey and for the same it is essential to document the compounds present in the honey found in several regions of India. Tylosine tartarate, erythromycin adihydrate, 4-epichlor otetracycline, enrofloxacin, sulfaqui noxaline, sulfachl oropyridazine are a few among the compounds predominate in honey. Chromatography-tandem mass spectrometry (UPLC-MS/MS) is being used to determine the compounds 3-amino-2-ozaxolidinone (AOZ, the marker residue for furazolidone), 3-amino-5-morpholino-methyl-1,3-oxa-zolidinone (AMOZ, the marker residue for furaltadone), 1-aminohydantoin (AHD, the marker residue for nitrofurantoin), and semicarbazide (SEM, the marker residue for nitrofurazone) present in the honey in one single analysis.

Keywords: Honey, UPLC, UPLC-MS/MS, 3-amino-2- ozaxolidinone, 1-aminohydantoin, antibiotics

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INTRODUCTION

Honey has been used for different purposes since ancient period of time and medicinal properties can be considered as the most important indigenous property of honey. Approximately 67 types of antibiotics are found in honey and most of them are required different analytical method to be determined [1, 2]. However, to accommodate the increasing demand of quality honey, the beekeepers are carrying out bad practices to increase the production and therefore, the quality of the honey often found degraded [3]. Additionally, contamination of honey and honeycomb are another crucial factor needed to be considered as the side effect of bad practices. Residues of different antibiotics and pesticides arethe major contaminants of honey which are often found carcinogenic and eventually affects the public health to a great extent. So far, there are no maximum residue limits (MRLs) for antibiotic residues in honey. Therefore, the presence of veterinary drugs in honey is not authorized [4, 5]. Various validated methods have been reported to determine the traces of antibiotic residues in honey and the method significantly depends on the sensitivity of the process. Chromatographic techniques, coupled with mass spectrometry, have turn out to be very popular in recent years. However, this method has limitation as the antibiotic compounds in honey are comprised of polar and non-polar compounds and developing a generalised method for determining both type of substances is quite challenging. In reversed phase liquid chromatography, it also exhibits almost negligible retention, apart from an ion-pairing reagent added to the mobile phase, also taking into account the appropriate quantity to lessen ionisation suppression [6, 7].

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Materials And Methods

3 gm of honey was collected and dissolved it into 5ml ofwater and subsequently 10ml of acetonitrile (ACN) was added and vortexed and finally centrifuged at X10,000 rpmfor 15 mins. The supernatant was collected and dried withN_2 evaporator. Finally, the sample again reconstitute with1ml ACN and water mixture (ACN:H_2O=1:1) and finallyfiltered with 0.2-micron nylon membrane. The trace ofantibiotics has been determined using UPLC-MS/MS (Model: AGILENT TQD 6850) where 5.00 µL of the sample was injected with the help of sampler (Model:

Table 1 : Solvent composition in gradient mode

Time (min)	*A (%)*	*B (%)*	*C (%)*
Start. Cond. min	95.00 %	5.00 %	0.00 %
1.00 min	95.00 %	5.00 %	0.00 %
4.00 min	70.00 %	30.00 %	0.00 %
12.00 min	2.00 %	98.00 %	0.00 %
14.00 min	2.00 %	98.00 %	0.00 %
14.10 min	95.00 %	5.00 %	0.00 %
16.00 min	95.00 %	5.00 %	0.00 %

Table 2 : Source parameter of MS

Parameter Id	*Positive Value*	*Negative Value*
Gas Temperature (°C)	300	300
Gas Flow (L/min)	8	8
Nebulizer (psi)	40	40
Capillary Voltage (V)	4000	4000
Sheath Gas Temperature (°C)	350	350
Sheath Gas Flow (L/min)	11	11
Nozzle Voltage (V)	1000	1000

G7129C) with an eject speed of 400 µL/min. Quaternary Pump is used for this purpose with a flow rate of 0.300 mL/min. The comumn temperature was maintained at 40˚C. Water and acetonitrile have been used in gradient mode and the same is represented in Table 1 [8, 9].

Finally, QQQ Mass Spectrometer (Module: Ultivo) was used which was equipped with AJS ESI ionization source and the operating parameters are given in Table 2 [10-12].

RESULTS AND DISCUSSION

The method developed in this current study are examined for quantitative determination of reference antibiotics and some of the chromatograms are represent in Fig. 1 (AMOZ) and 2 Semicarbazide (SC).

In this study, the single-run UPLC-MS/MS method was developed to quantify antibiotics in honey. The chromatograms present multiple details related to retention times, specificity, sensitivity, fragmentation pattern, applicability etc. The chromatograms for AMOZ and SC present the peaks at 2.952 minutes and 5.260 minutes respectively. Two different retention times confirms the ability of the method for distinguishing each antibiotic. The

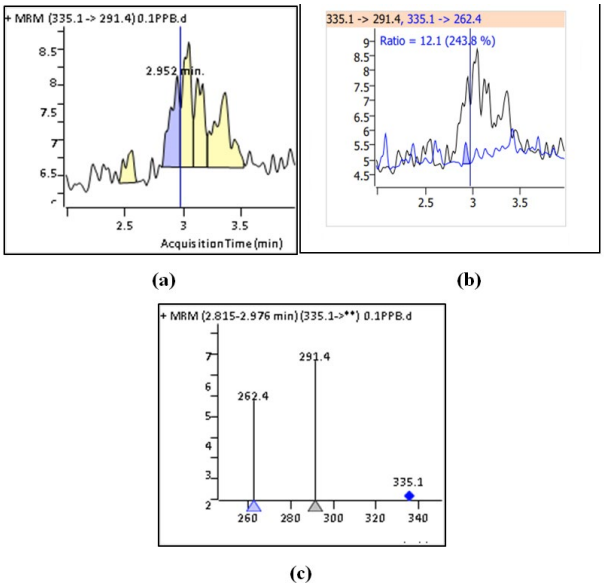

Fig. 1: Chromatogram of AMOZ

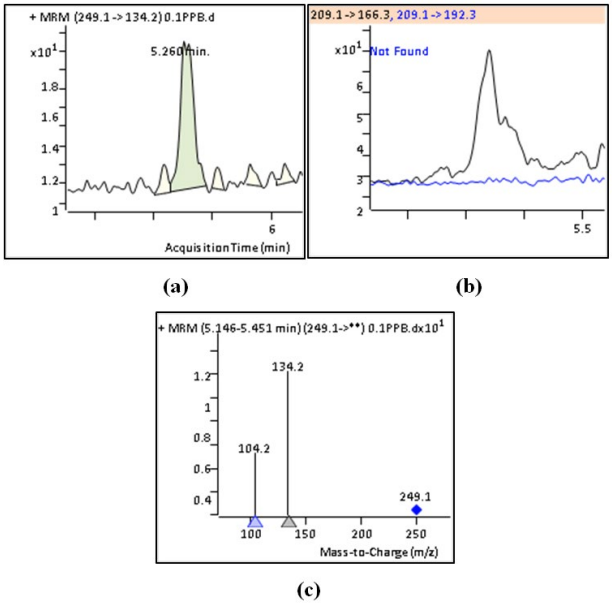

Fig. 2: Chromatogram of Semicarbazide (SC)

Table 3 : Standard Calibration for AMOZ

Level	*Enabled*	*Resp.*	*Exp. Conc*	*Resp. Factor*
1	x	14	0.1000	139.0112
2		212	1.0000	212.2469
3	x	5302	10.0000	530.2313
4	x	55749	100.0000	557.4942
5	x	524044	1000.0000	524.0443

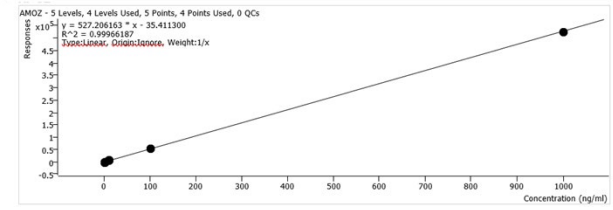

Fig. 3: Linearity plot of AMOZ

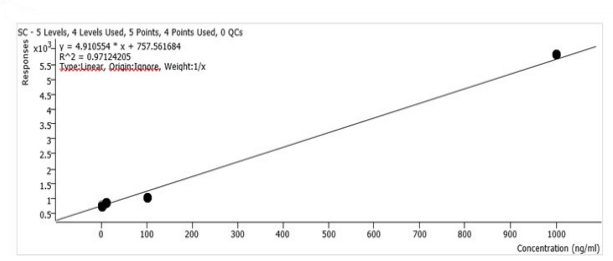

Fig. 4: Linearity plot of Semicarbazide (SC)

Table 4 : Standard Calibration for SC

Level	*Enabled*	*Resp.*	*Exp. Conc*	*Resp. Factor*
1	x	758	0.1000	7577.5143
2		774	1.0000	774.3937
3	x	857	10.0000	85.7474
4	x	1024	100.0000	10.2450
5	x	5842	1000.0000	5.8417

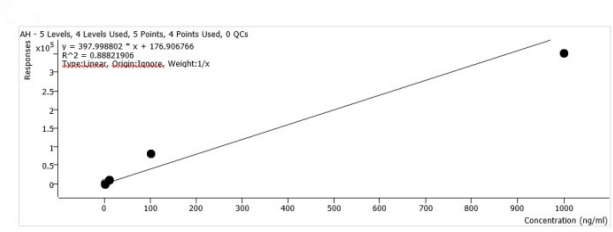

Fig. 5: Linearity plot of 1-aminohydantoin (AH)

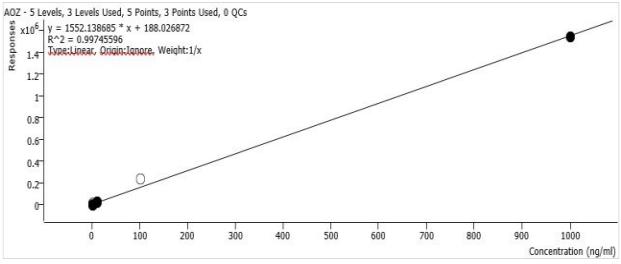

Fig. 6: Linearity plot of AOZ

Table 5 : Standard Calibration for AH

Level	*Enabled*	*Resp.*	*Exp. Conc*	*Resp. Factor*
1	x	126	0.1000	1261.6375
2		931	1.0000	931.2093
3	x	9560	10.0000	956.0396
4	x	81131	100.0000	811.3142
5	x	351708	1000.0000	351.7081

Table 6 : Standard Calibration for AOZ

Level	*Enabled*	*Resp.*	*Exp. Conc.*	*Resp. Factor*
1	x	266	0.1000	2660.2624
2		2408	1.0000	2407.7309
3	x	23508	10.0000	2350.8079
4		230037	100.00	2300.3742
5	x	1544605	1000.0	1544.6053

method is also capable of detecting flow concentrations of AMOZ and SC by achieving a sensitivity level that meets the quantification needs for honey samples. It becomes clear that the UPLC-MS/MS method is effective for screening multiple antibiotics in a single run.

Additionally, the linearity has been developed using the reference antibiotics at different concentration points and the sane has been represented below.

From the table of standard calibration for AMOZ, it is identified that the response factor is increasing studies with the increase of the expected concentration. It indicates that there is a positive and linear relationship between concentration and response. On the other hand, the standard calibration for SC so that there is a negative and nonlinear relationship between concentration and response for it. It is because the response factor is decreasing significantly with the increase of concentration. Similar types of nonlinear relationships are also visible for AH and AOZ.

CONCLUSION

At the conclusion, it can be stated that here we have developed a Single-Run method in which near about 36 Antibiotics that belongs from different categories can be detected. This is not an analysis with higher concentration level, this has been developed at very low level of concentration like 0.5- 1 PPB with a single Mass Method. This is a robust as well rugged method with a minimal deviation of less than 5% and the slopes of the linearity are

more that 0.9; this also indicates it a validated method. By this the analysis of antibiotics by Mass Spectroscopy will be much more easier and time consuming also. Besides, this also helps to reduce the analysis cost as well as helps to retain the performance of the column and the detector.

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Conflict Of Interest

Authors declare no conflict of interest related to the study.

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