ION LABS	Standard Operating Procedure **Raspberry Ketone and Zingerone Determination by HPLC using UV/VIS Spectroscopy**	**SOP Number** **D-727**	**Revision** **6**
		Effective Date 06/29/22	**Page** **Page 1 of 8**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
SAS 06/02/22	06/14/22	06/20/22
Title: Analytical Development Scientist	**Title: Analytical Development Manager**	**Title: QC Laboratory Director**

1.0 Purpose

The purpose of this procedure is to define a method for the quantitative analysis and/or identification of raspberry ketone (4-(4-hydroxyphenyl)-butanone) and zingerone (vanillylacetone) in raw materials and finished products using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification and identification of raspberry ketone and zingerone. Some excipients and dietary ingredients used in finished products may interfere with the analysis of raspberry ketone or zingerone.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory and Analytical Development Management to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **Raspberry Ketone** – 4-(4-hydroxyphenyl)-2-butanone

4.2 **Zingerone** – Vanillylacetone or 4-(4-hydroxy-3methoxyphenyl)-2-butanone

4.3 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums

4.4 **H_3PO_4** – Phosphoric Acid

4.5 **CofA** – Certificate of Analysis

4.6 **H_2O** – Water

4.7 **QC** – Quality Control

5.0 References

5.1 MV-LAB-12-009, Protocol, Raspberry Ketone 4(4-Hydroxyphenyl)-2Butanone Determination by HPLC

5.2 MV-LAB-18-067, Protocol, Zingerone Determination by HPLC using UV/VIS Spectroscopy

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents: all reagents are HPLC or better.

6.1.1 H_2O ($\geq$ 18.2 MΩ·cm)

6.1.2 Acetonitrile

6.1.3 Methanol

6.1.4 H_3PO_4

6.1.5 Raspberry Ketone reference standard

6.1.6 Zingerone reference standard

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 1L mobile phase container

6.2.3 10mL, 50mL, 100mL, 500mL, and 1L volumetric flasks

6.2.4 200µL, 1mL, and 10mL pipette tips

6.2.5 10mL Plastic luer-lock syringes

6.2.6 0.45µM Nylon syringe filters

6.2.7 22mL screw cap vials

6.2.8 1.5mL and 2.0mL micro centrifuge tubes

6.2.9 Weigh paper and weigh boats

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Column - 5µm, C5, 150mm X 4.6mm

6.3.3 Analytical Balance

6.3.4 Stir Plate

6.3.5 Wrist Action Shaker

6.3.6 Vortex

6.3.7 Sonicator Bath

6.3.8 200µL, 1mL, and 10mL Pipettes- adjustable

7.0 Procedure

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase A (0.1% H_3PO_4 in H_2O)

7.1.1.1 Transfer 1000 mL of H_2O to a 1000-mL mobile phase bottle.

7.1.1.2 Add 1.0 mL H_3PO_4, and mix well.

7.1.2 Mobile Phase B (0.1% H_3PO_4 in acetonitrile)

7.1.2.1 Transfer 1000 mL of acetonitrile to a 1000-mL mobile phase bottle.

7.1.2.2 Add 1.0 mL H_3PO_4, and mix well.

7.1.3 Diluent (100% methanol)

7.2 Standard Preparation

7.2.1 The linear range of the method is listed below. All standard and sample preparations must be within the linear range.

7.2.1.1 Raspberry Ketone – 0.0125 to 0.25 mg/mL

7.2.1.2 Zingerone – 0.01 to 0.1 mg/mL

7.2.2 Use the actual purity from the CofA for raspberry ketone or zingerone reference standard in your calculations. The Standard Preparation reflects 100% of the label quantity for raspberry ketone and zingerone.

7.2.3 All standards are prepared by weighing no less than the minimum weight of the analytical balance. Dissolve in and dilute to volume in an appropriately sized volumetric flask using Diluent.

7.2.4 Dilutions can be made using volumetric flasks or using 1mL and 200uL variable pipettes. Working standard concentrations will approximate the concentration expected to be found in the product being tested based on the sample dilution and calculated from the label. Final dilutions may be prepared directly in HPLC vials.

7.3 Sample Preparation

7.3.1 At least 20 dosage units are pooled and ground by mortar and pestle as necessary.

7.3.2 Samples are prepared by weighing no less than the minimum weight of the analytical balance.

7.3.3 Samples can be dissolved in Diluent at any volume starting from 25mL. To manage large volumes the sample can be initially dissolved in a smaller volume and a portion further diluted to bring the analyte concentration into the linear range of measurement.

7.3.4 Based on the label claim and fill or tablet weight per dose for finished products or expected potency for raw materials, weigh a portion of the sample into a suitably sized volumetric flask to generate an analyte concentration that is within the validated linear range for the analyte being tested.

7.3.5 Dilute the sample to 2/3 of the flask volume with Diluent, and shake for 20 minutes to facilitate dissolution. Sonication for 10 minutes can also be used to assist dissolution. Once the analyte is completely dissolved, bring sample up to volume with Diluent before any further dilutions.

7.3.6 If sonication is used, allow sample to cool to RT before continuing.

7.3.7 For filtration, using the final large scale diluted sample withdraw up to 10mL using a 10mL plastic syringe then filter and discard at least 0.5mL of sample before collecting. From the collected sample dilute as needed then add 1mL to an HPLC vial for analysis.

7.3.8 For centrifugation using the final large scale diluted sample, fill an even number of 1.5 or 2.0mL micro-centrifuge tubes and pellet insoluble matter for 5 minutes at 6000rpm.

7.3.9 For finished products or raw materials being analyzed for the first time using this method an in process validation is required to demonstrate spectral purity, baseline separation of peaks and extraction efficiency as a part of system suitability before data can be reported using this method.

7.4 Test Conditions

7.4.1 Gradient - Isocratic 65% Mobile Phase A : 35% Mobile Phase B

7.4.2 Column - 5µm, C5, 100Å, LC column, 150mm X 4.6mm

7.4.3 Flow Rate - 1.0 mL/min

7.4.4 UV Detection - 280nm

7.4.5 Injection Volume - 20µL

7.4.6 Temperature - 45°C

7.4.7 3-D Spectral Range- 200nm to 350nm

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of a Blank (Diluent).

7.5.2 Make five injections of the Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

7.5.4 Make a single injection of the Working Standard after every six samples and at the end of the run.

7.6 System Suitability

7.6.1 The %RSD of five consecutive injections of Working Standard is NMT 5.0%.

7.6.2 The %RSD of all Working Standard injections is NMT 5%.

7.7 Column Wash and Storage

7.7.1 Rinse the column with H_2O / ACN (50/50) at 1 mL/min for at least 15 min.

7.7.2 Store the column with H_2O / ACN (50/50).

8.0 Example Calculations

8.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

R_u Sample peak area

R_s Mean standard peak area

Wt_{std} Weight of reference standard in mg

V_{std} Volume of the standard preparation accounting for dilutions in mL

P Purity of the reference standard in decimal format

SA Sample amount in mg (solids) or mL (liquids)

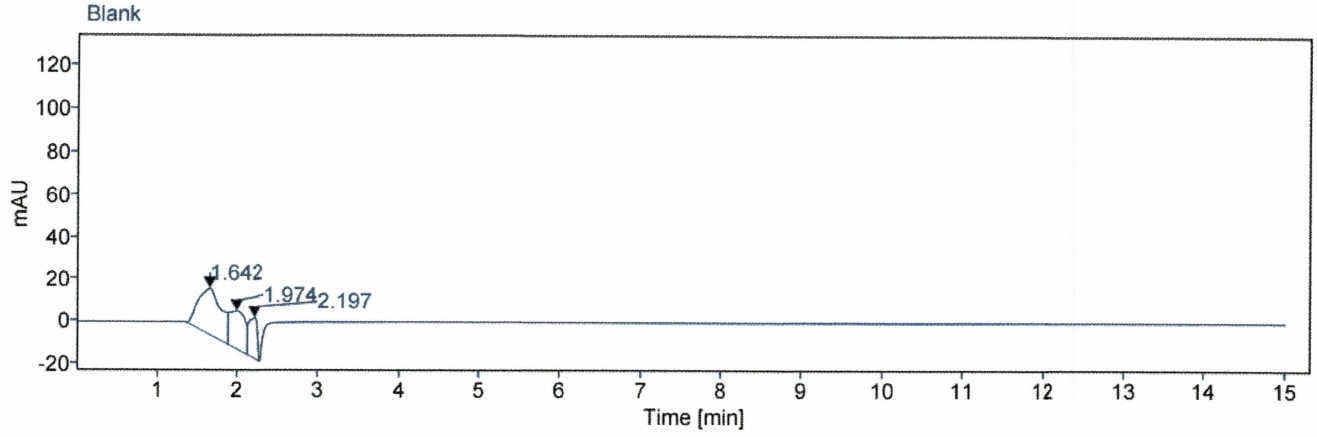
V_{spl} Volume of the sample preparation accounting for dilutions in mL

SS Serving size: Weight of a single dosage unit in mg for tablets and capsules, volume of a single serving from the theoretical formula in mL for liquids, or 1 for raw materials.

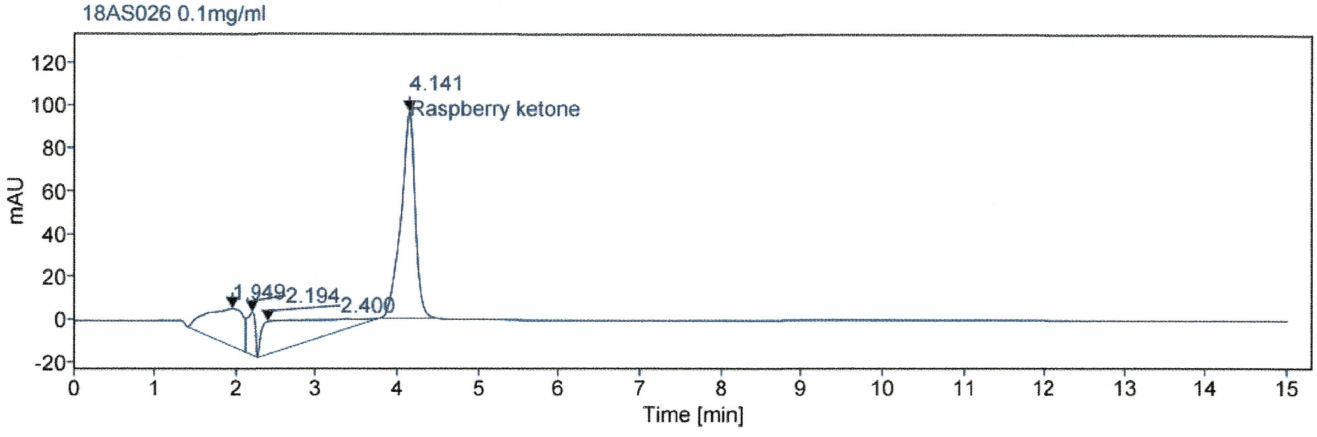
LA Label amount in mg per dose or 1 for raw materials

9.0 Example Chromatography

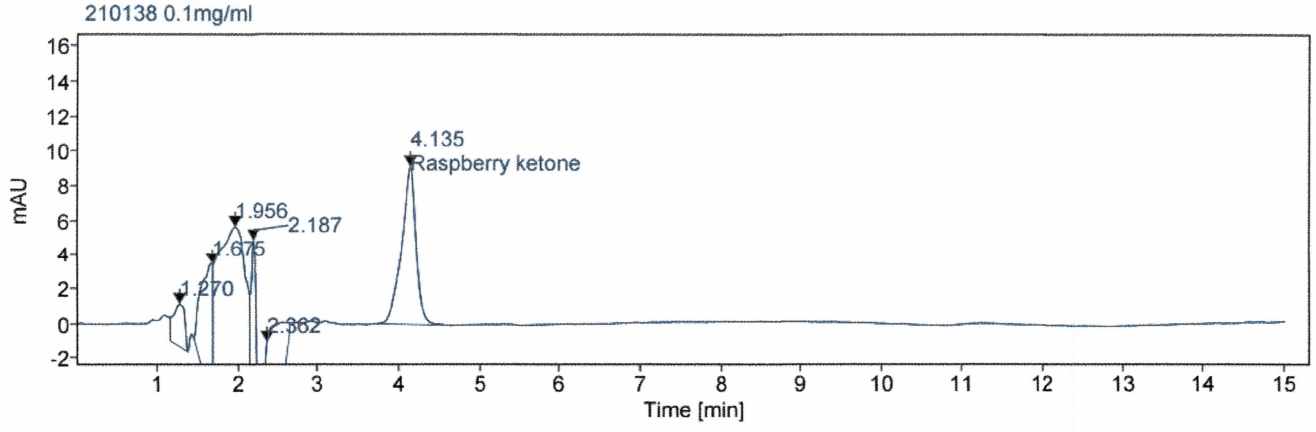
9.1 Blank



9.2 Working Standard



9.3 Sample



10.0 Revision History

Revision	Date	Description of Changes	CCR #	By
1	12/17/12	New procedure.		B. Johns
2	04/03/13	Updated SOP logo and name, added new calculations, added detailed information on sample and standard preparation, added Attachment 1		B. Johns
3	11/18/13	Updated format, removed redundant information, added new calculation for standard preparation, added dissolution buffer description, error correction	13-1055	B. Johns
4	04/01/16	Biennial review: Updated SOP to current format.	16-0203	J. Maignan
5	01/03/19	Updated SOP. Added zingerone based on validation.	19-0007	J. Maignan
6	06/01/22	Update for consistency with current methods, add recommended sequence section, replace requirements with system suitability section, add example chromatography.	CC-22-0251	S. Sassman