



**International
Standard**

ISO 21114

**Nicotine pouches — Determination
of nicotine content using liquid
chromatography ultraviolet
spectroscopy (LC-UV)**

*Sachets à base de nicotine — Détermination de la teneur en
nicotine par chromatographie en phase liquide couplée à la
spectroscopie ultraviolette (LC-UV)*

**First edition
2026-10**

Sample Document

get full document from standards.iteh.ai



COPYRIGHT PROTECTED DOCUMENT

© ISO 2026

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Apparatus	1
6 Reagents	3
7 Preparation	3
7.1 Preparation of glassware	3
7.2 Preparation of solutions	3
7.2.1 Ammonium formate, 500 mmol/l (used for preparing extraction solution (7.2.2))	3
7.2.2 Extraction solution, 100 mmol/l ammonium formate	3
7.2.3 Ammonium acetate stock buffer, 500 mmol/l (used for mobile phases)	4
7.2.4 Mobile phase A, 10 mmol/l ammonium acetate buffer + 0,025 % ammonium hydroxide	4
7.2.5 Mobile phase B, 10 mmol/l ammonium acetate buffer in acetonitrile + 0,025 % ammonium hydroxide	4
7.2.6 Needle wash, 40:40:20:2 of water:methanol: 2-propanol:formic acid	4
7.2.7 Seal wash, 90:10 of water:acetonitrile	4
7.3 Preparation of standards	4
7.3.1 Nicotine standard stock solution: approx. 25 mg/ml	4
7.3.2 Nicotine calibration standards	4
8 Sample preparation and extraction	5
8.1 General	5
8.2 Sample preparation	5
8.3 Sample extraction	5
9 Sample extract analysis	6
9.1 General	6
9.2 Suggested UPLC parameters	6
9.3 Suggested HPLC parameters	7
9.4 System suitability	7
9.5 Calibration	7
10 Repeatability and reproducibility	8
11 Test report	9
Annex A (informative) Examples of UPLC chromatograms	10
Annex B (informative) Examples of HPLC chromatograms	12
Bibliography	14

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Nicotine pouches are portioned consumer products that do not contain cut, ground, powdered or leaf tobacco, but do contain nicotine, a base filler, and material may contain flavours, sweeteners, pH adjusting agents and other ingredients. Nicotine pouches are intended for oral use and uptake of nicotine via the oral mucosa. The accurate determination of nicotine is an important analytical measurement for product characterization.

In 2024, ISO/TC 126 for nicotine pouches conducted a large international collaborative study with 23 participating laboratories. The method allows the use of ultra performance liquid chromatography (UPLC) and high performance liquid chromatography (HPLC). For the collaborative study, it was agreed that the laboratories can submit data using both HPLC and UPLC under the requirement that a separate sample extraction was prepared for each technique. In total, 27 datasets were submitted, including 17 HPLC data sets and 10 UPLC data sets. This study data was used as the basis for calculating the repeatability (r) and reproducibility (R) limits estimates for this method. As agreed prior to the start of the study, the data from both HPLC and UPLC were examined to evaluate if the two techniques gave similar results and performance. It was concluded that both HPLC and UPLC results were similar, and that the data can be combined to determine the r and R limits. Results from this study were evaluated in basic conformance with ISO 5725-2 to calculate repeatability and reproducibility values.

Sample Document

get full document from standards.iteh.ai

Sample Document

get full document from standards.iteh.ai

Nicotine pouches — Determination of nicotine content using liquid chromatography ultraviolet spectroscopy (LC-UV)

WARNING — The use of this document can involve hazardous materials, operations, and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of any other restrictions prior to use.

1 Scope

This document specifies a method for the determination of nicotine content of nicotine pouches and nicotine pouch filler using HPLC/UPLC with UV spectroscopy.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes the requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

nicotine pouch

portioned consumer product that does not contain cut, ground, powdered, or leaf tobacco but does contain nicotine, a base filler material, and may contain flavours, sweeteners, and pH adjusting agents, and other ingredients

Note 1 to entry: Nicotine pouches are intended for oral use and uptake of nicotine via the oral mucosa.

4 Principle

Nicotine is extracted from nicotine pouch/nicotine pouch filler sample with a buffer solution (pH 3). The extracts are filtered and analysed by HPLC/UPLC with UV spectroscopy. Nicotine is reported for pouch as mg/pouch or mg/g and for filler as mg/g, on an as is basis.

The method calibration range is (0,025 to 0,5) mg/ml or (2 to 40) mg/g.

5 Apparatus

Usual laboratory apparatus, and particularly the following items.