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A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DEXLANSOPRAZOLE IN BULK AND EXTENDED-RELEASE CAPSULES

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ABSTRACT

A simple, precise, specific, and accurate RP-HPLC method has been developed and validated for the estimation of Dexlansoprazole in bulk and capsule dosage formulation. The separation was achieved by Enable C18column (250X 4.6mm, 5µm particle size) using a mobile phase consisting of methanol and water (95:5, v/v) at a flow rate of 1mL / min using detection wavelength at 247nm. The method was developed in isocratic mode. The retention time was around 2.914mins. The method showed linearity with correlation coefficient R^2=0.997 over the range of 20-120µg/mL. The mean recoveries were found to be in the range of 99.3-99.7% for Dexlansoprazole. The method was validated as per ICH guidelines for linearity, the limit of Detection, the limit of quantification, accuracy, precision, and robustness, ruggedness. The method can be successfully applied for routine analysis of the quantitative determination of Dexlansoprazole in the pharmaceutical dosage form.

KEYWORDS: Dexlansoprazole, RP-HPLC, Method development, validation, ICH.

INTRODUCTION

Dexlansoprazole chemically is (R)-(+) 2-([3-methyl-4-(2, 2, 2-trifluoromethoxy) pyridin-2yl] methyl sulfinyl)-1H-benzimidazole (Fig. 1). It is a proton pump inhibitor.[1] by the healing of Erosive Esophagitis and symptomatic Non-Erosive Gastroesophageal Reflux Disease.[2] Dexlansoprazole is the R-enantiomer of lansoprazole (a racemic mixture of the R- and S- enantiomers). It is a white powder having a molecular formula $C_{16}H_{14}F_3N_3O_2S$ and a molecular weight of 369.363. It is freely soluble in methanol. The mechanism action of dexlansoprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific

inhibition of the (H+, K+)-ATPase in the gastric parietal cell. By acting specificity on the proton pump, Dexlansoprazole blocks in the final step of acid production.

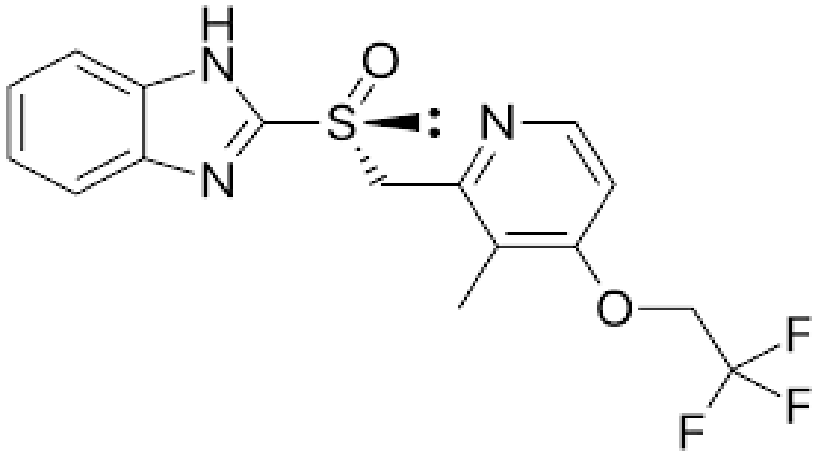

Figure 1: Chemical structure of Dexlansoprazole.

A detailed literature survey for Dexlansoprazole revealed that a very few UV spectrophotometric, HPLC and LCMS.[3,6] methods have been reported for the quantification of Dexlansoprazole. Hence an attempt was made to develop a new simple, precise, accurate, isocratic mode RP-HPLC method for the determination of Dexlansoprazole in Bulk and Capsules.

Dexlansoprazole was approved by US-FDA in 2010 with a brand name DELTONE[7]. The developed method was validated as per ICH Guidelines.[8,9]

MATERIALS AND METHODS

Chemicals and reagents

A Dexlansoprazole with a purity of 100.1% w/w was obtained as a gift sample and formulation of Dexlansoprazole (Deltone®) was procured from the local market, chemicals and reagents used are of analytical grade. Chemicals like methanol and water are of HPLC grade obtained from Merck Life sciences Pvt Limited.

Instruments and Apparatus

ELITE analytical balance and Shimadzu HPLC instrument (LC-20AD) with a binary pump, with UV – Visible detector was used. An enable C18 column (250 X 4.6mm, 5µm particle size) was used. Rheodyne injector with a 20 µL loop was used and data was recorded using LC solution software.

Chromatographic conditions

Accurately weighed 100mg of drug and was transferred into 100 ml clean dry volumetric flask the contents were dissolved using methanol and sonicated for 15mins, later the volume was made up using methanol. Further 10ml from the above stock solution was transferred to 100ml volumetric flask and made up to mark with methanol to get a concentration of 100µg/ml.

In setting up the optimized conditions for development, the choice of detection wavelength was based on the scanned absorption spectrum for the UV- the spectrum of Dexlansoprazole, which was obtained by scanning the sample over the wavelength range of 200-400nm against blank as diluent. After a thorough examination of the spectra, the wavelength 247nm was selected for further analysis.

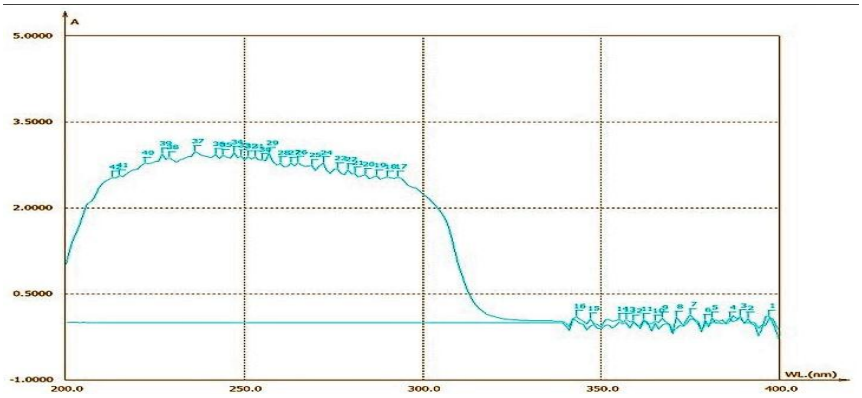

Figure 2: Determination of λ_{max} of Dexlansoprazole.

Preparation of mobile phase

A mixture of Methanol and water in the ratio of 95:5 was prepared and sonicated for 30 minutes to remove gases.

Preparation of standard solution

A standard drug solution of Dexlansoprazole was prepared by adding 100 mg of the drug into a 100 mL volumetric flask and made up to mark with methanol to get a concentration of 1000 µg/mL.

Preparation of working standard solution

From the above standard stock solution, 6 mL of sample was transferred to 100 mL volumetric flask and made up to mark with methanol to get a concentration of 60 µg/mL.

Preparation of sample solution

The proposed method was applied to analyze the commercially available Dexlansoprazole capsules Deltone ® (60mg). 20 capsules were weighed and powdered, the amount equivalent to 100mg of Dexlansoprazole was weighed accurately and transferred into 100 mL volumetric flask containing methanol which was further sonicated for 30 min with vigorous shaking, the volume was brought up to 100mL with methanol. The solution was subjected to filtration through Whattman filter paper #44. The filtrate was diluted suitably with methanol to get a final solution of 60µg/ml concentration. This was subsequently analyzed using a UV-VIS detector the chromatogram was recorded at 247 nm.

Table 1: Optimized chromatographic conditions.

HPLC Instrument	**Shimadzu LC solutions Software**
UV-Visible Spectrophotometer	Lab India (T60)
Column	Enable C18 column (250 X 4.6mm, 5µm particle size).
Mobile phase	Methanol: water (95:5)
Flow rate	1mL/min
Detection wavelength	247nm
Run time	5 min
Retention time	2.914 min

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity, will consistently produce the desired result, meeting its predetermined specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, Range, Sensitivity and Selectivity.[10,13]

RESULTS AND SISCUSSION

System suitability

System suitability was determined from six replicate injections of the standard solution before the analysis and the chromatograms were recorded. HPLC system is allowed to stabilize for 30mins, injected blank preparation (single injection), and standard preparation (six replicates), %Relative standard deviation (%RSD) for six replicates of the standard was calculated. System suitability parameters like symmetry, theoretical plates, and tailing factors were also recorded. The system suitability data are reported in [Table 2].

Table 2: System Suitability Parameters.

Parameters	Dexlansoprazole
Retention time	2.914
Peak area	1313934
Theoretical plates	26322.795
Tailing factor	1.159

Specificity

Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix such as impurities or excipients. There should not be any interference of the diluents or placebo at the retention time of drug substances.

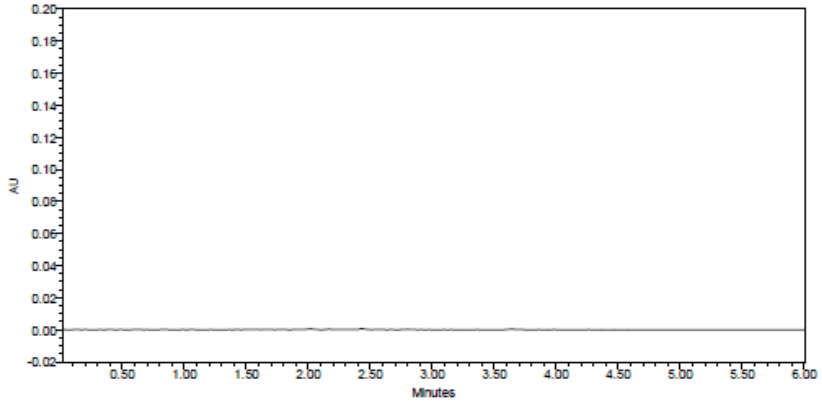

Figure 3: Chromatogram of Blank.

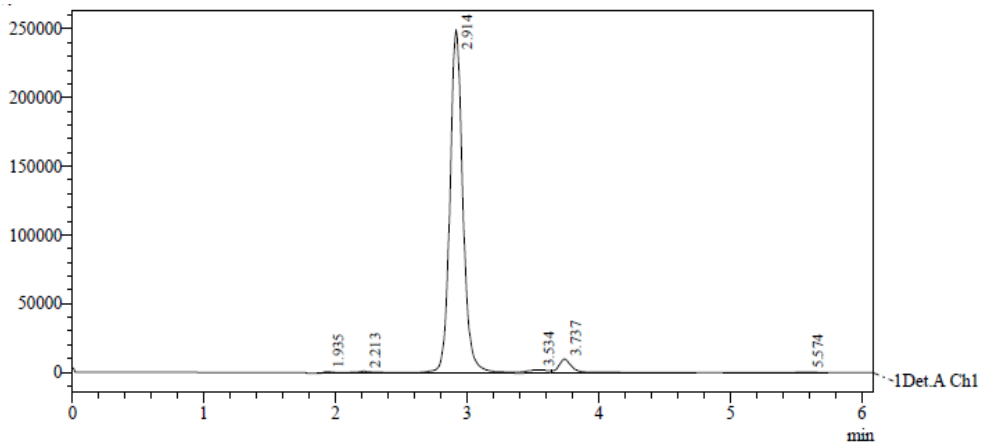

Figure 4: Chromatogram of standard.

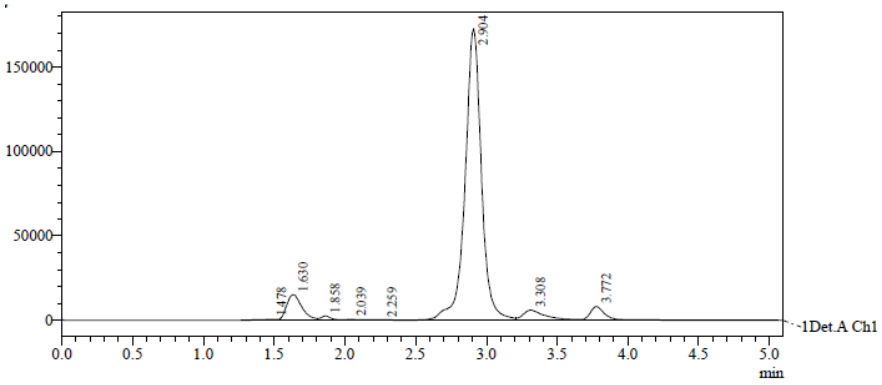

Figure 5: Chromatogram of sample.

Table 3: Specificity results for standard and sample.

Parameters	Dexlansoprazole standard	Dexlansoprazole sample
Retention time (min)	2.914	2.904
Number of Theoretical plates (N)	26322.795	20623.100
Tailing factor (T)	1.159	0.955
Area	1313934	1299563

Observation

It is observed from the above data, diluents or excipients peaks are not interfering with the Dexlansoprazole peak.

Linearity

Different aliquots of Dexlansoprazole were prepared from the working standard solution (100µg/mL) in the range of 20-120µg/mL respectively. Calibration curve showing concentration Vs peak area was plotted [Table 4].

Table 4: Linearity data for Dexlansoprazole

Concentration(µg/mL)	Peak area
20	367114
40	820665
60	1198060
80	1701025
100	2210564
120	2587988

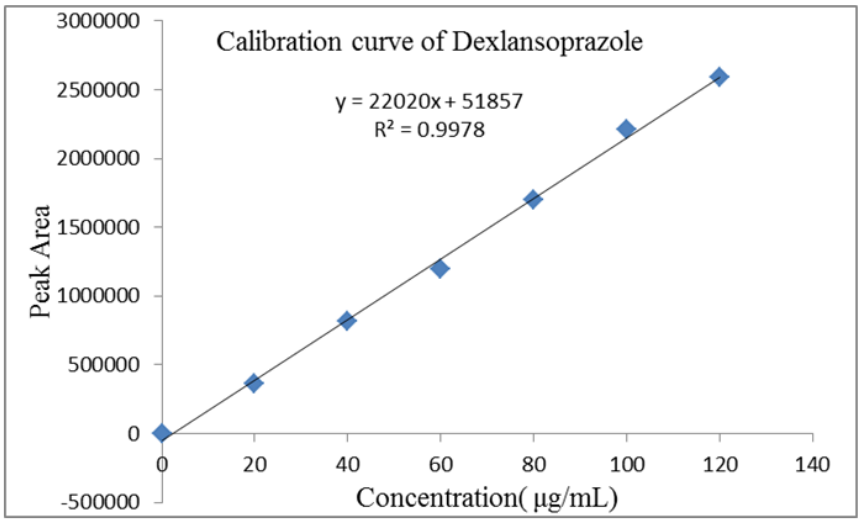

Figure 6: Calibration of Dexlansoprazole.

Observation

The correlation coefficient for the linear curve obtained between the concentration Vs Peak area was found to be 0.9978.

Precision

The precision of the method was demonstrated by an inter-day variation study. In the inter-day variation study, the solutions of the same concentration 60µg/mL,40µg/mL, 80µg/mL were prepared and analyzed six times, for two consecutive days, and the peak area were reported [Table 5, 6, 7].

Table 5: Inter-day precision (60µg/mL).

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
60	1107423	1114588
60	1109925	1115226
60	1108543	1119626
60	1100571	1119563
60	1106452	1114542
60	1102458	1108629
	Avg: 1105895 **SD**: 3634.715 **%RSD**: 0.328%	**Avg**: 1115362 **SD:** 4060.871 **%RSD:** 0.364%

Table 6: Inter-day precision (40µg/mL).

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
40	838699	824490
40	829058	836843
40	825549	835476
40	827287	838062
40	827522	831088
40	827966	835214
	Avg: 829346.8 **SD**: 4721.22 **%RSD**: 0.569%	**Avg**: 833528.8 **SD:** 5016.427 %**RSD:** 0.60183%

Table 7: Inter-day precision (80µg/mL).

Concentration (µg/mL)	Peak Area (Day-2)	Peak Area (Day-1)
80	1785328	1880368
80	1786053	1858642
80	1780863	1864390
80	1770321	1855062
80	1783248	1858302
80	1785535	1834934
	Avg: 1781891 **SD**: 5986.26 **%RSD**: 0.335%	**Avg**: 1858616 **SD:** 14697.3 %**RSD:** 0.790%

Observation The % RSD for the area of 6 injections was found to be less than 2. Hence the results obtained were found to be satisfactory.

Accuracy

The accuracy of the method was determined by preparing solutions of three different levels, i.e., 80, 100, and 120%, in which the amount of marketed formulation Deltone ® was kept constant (60µg/L) and the concentration of pure drug was varied, that is 48µg, 60µg, and 72µg for 80, 100, and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery and was calculated and reported in the [Table 8].

Table 8: Accuracy data.

Level of Addition (%)	Amount added (µg/mL)	Drug found (µg/mL)	% Mean Recovery
80%	48	47.66	99.31
100%	60	59.6	99.30
120%	72	71.76	99.70

Observation

The percentage mean recovery of Dexlansoprazole was found to be 99.43%.

Limit of detection and limit of detection

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of the y-intercept and slope of the calibration curve by using the following formulas.

Limit of detection = 3.3 × standard deviation / slope.

Limit of quantification = 10 × standard deviation / slope.

Table 9: LOD and LOQ values.

Parameters	µg/ml
Limit of detection	0.49µg/ml
Limit of quantification	1.65µg/ml

Robustness

The robustness of an analytical method was the measurement of its capability to remain unaffected by small but deliberate variations in the method parameters. Robustness was done by changing the flow rate (±0.1ml/min) and wavelength (±1nm). The results were reported [Table 10].

Table 10: Robustness results for Dexlansoprazole

Parameter	Theoretical plates	Tailing factor
Flow rate		
0.9 mL/min	20935.688	0.870
1.1 mL/min	19853.131	0.882
Wave length		
246nm	22140.535	0.980
248 nm	21675.992	0.928

Observation From the observation it was found that the system suitability parameters were within the limit at all variable conditions.

Ruggedness

Ruggedness is termed by the degree of reproducibility of results by analyzing the same sample under a variety of conditions, such as different laboratories, analysts, and instruments. The method was studied by two different analysts. The results were reported [Table 11].

Table 11: Ruggedness data.

Concentration (µg/mL)	Peak Area (Analyst-1)	Peak Area (Analyst-2)
60	1114588	1107423
60	1115226	1109925
60	1119626	1108543
60	1119563	1100571
60	1114542	1106452
60	1108629	1102458
	Avg: 1115362 **%RSD**: 0.364%	**Avg**: 1105895 **%RSD:** 0.328%

From the observation, %RSD between two analysts were not greater than 2%, hence the method was found to be rugged.

Assay

The percentage purity of Dexlansoprazole capsules (Deltone®, 60mg) was found to be 98.9%.

RESULTS AND DISCUSSION

The present study was aimed to develop a novel, sensitive, precise, accurate HPLC method for the estimation of Dexlansoprozole in the bulk and pharmaceutical dosage form. In order to achieve an optimum separation of the component peak, the mixture of methanol and water in the ratio 95:5 as a mobile phase on an Enable C18 stationary phase. A binary mixture of methanol: water in the ratio 95:5 v/v was selected as the chromatographic peaks were well defined and resolved with less tailing. The retention time obtained for Dexlansoprazole was 2.910 min. Each of the samples was injected six times and the same retention times were observed in all cases. The peak areas of Dexlansoprazole were reproducible as indicated by the low coefficient of variation. A good linear relationship (r^2 = 0.997) was observed between the concentration of Dexlansoprazole and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was be y=22020x+51857. Intra-day and Inter-day precision was carried out for the proposed method and was found to be precise as the %RSD value was less than 2. High recovery values obtained from three different levels, as the proposed method was checked by recovery studies. The high recovery values indicate the accuracy of the developed method. The deliberate changes in the method have not much affected the peak tailing, theoretical plates, and retention time. This indicates the robustness of the method. The lowest value of LOD and LOQ obtained by the proposed method indicates the sensitivity of the method.

All the results obtained are represented in [Table 12].

Table 12: Summary of Validation Parameters for the proposed method.

Parameters	Results
Absorption maxima (nm)	247
Linearity range (µg/mL)	20-120
Regression equation	y= 22020x+51857
Correlation coefficient (R^2)	0.997
LOD (µg/ml)	0.49
LOQ (µg/ml)	1.65
Accuracy (% Recovery)	99.30 - 99.70
Precision	
Inter-day precision (%RSD)	
Day-1 Day-2	0.328% 0.364%
Assay (%)	98.9

CONCLUSION

Hence, it can be concluded that the proposed HPLC method is simple, precise, and accurate for the determination of Dexlansoprazole in bulk as well as in the pharmaceutical dosage form. Moreover, The HPLC method enables faster quantification of Dexlansoprazole with an analysis time of three minutes without the interference of excipients; the proposed method can be used for routine quality control of pharmaceutical formulation containing Dexlansoprazole.

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CONFLICTS OF INTEREST

Authors do not have any conflict of interest.

REFERENCES

1. Nagaya H, Satoh H, Maki Y: Possible Mechanism For the inhibition of Acid Formation by Proton Pump Inhibitior AG-1749 in Isolated canine parietal cells. Journal of Pharmacology and Experimental Therapeutics, 1990; 252: 1289-1295.

2. Metz D, Vakily M, Dixit T: Dual Delayed Release Formulation of Dexlansoprazole MR, a novel approach to overcome the limitations of conventional single release proton pump inhibitor therapy. Alimentary pharmacology and therapeutic, 2009; 29(9): 928-937.
3. Hotha KK, Vijaya Bharathi D, Jagadeesh B, Ravindranath LK, Jaya Veera KN, Venkateswaralu V: Development and Validation of a Highly Sensitive LC-MS/MS Method For Quantitation of Dexlansoprozole in Human Plasma: Application to a human Pharmacokinetic study. Biomedical Chromatography, 2012; 26(2): 192-8.
4. Geetharam. Yanamadala, Praveen srikumar P, Rushyendra G.V, Ramamohanupta.V, Srinivasarao S: Stability Indicating Validated Novel RP-HPLC Method for the Estimation of Dexlansoprozole In Bulk And Extended-Release Capsules. Indo American Journal of Pharmaceutical Research, 2013; 3(10): 8457-8466.
5. Jalli Sriharsha, Srinivasa Murthy M, Bharat Kumar D, Sravan K, Shiva Kumar P, Shirisha A, Pranusha k: Method Development And Validation For Simultaneous Estimation of Dexlansoprozole and Meloxicam by RP-HPLC. Pharmaceutica Analytica Acta., 2015; 6(5): 1-3.
6. Balamurugan P, AnverBasha K, Jeenet Jayachandran, Manish Gangrade, Parthiban P: A Simple RP-HPLC Method For Simultaneous Estimation Of Organic Impurities, Enantiomer And Assay of Dexlansoprozole. International Journal of Pharmacy And Pharmaceutical Sciences, 2015; 7(9): 347-352.
7. Stability testing of new drug substances and products, Q1A (R2), in International Conference on Harmonization (ICH), International Federation of Pharmaceutical Manufacturers and Associations, Geneva, Switzerland, 2003.
8. International Conference on Harmonization, Validation of Analytical Procedure, Text and Methodology Q2 (R1), International Federation of Pharmaceutical Manufactures and Associations, Geneva, Switzerland, 2005.
9. Tangri Pranshu, Rawat Prakash Singh, JakhmolaVikash. Validation: A Critical Parameter for Quality Control of Pharmaceuticals. Journal of Drug Delivery & Therapeutics, 2012; 2(3): 34-40.
10. Validation of Analytical procedure Methodology ICH "Harmonized Tripartite Guidelines," 1996: 1-8.
11. ICH – Guidelines Q2 (R1), Validation of Analytical Procedure: Text and Methodology.
12. ICH- Guidelines Q2B, Technical Requirements for Registration of Pharmaceutical for Human Use Guideline on validation of Analytical Procedures-Methodology, Geneva, Switzerland.