ISSN: 2277-8713
Mitul Patel, IJPRBS, 2012: Volume1 (2): 274-286 *IJPRBS*

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

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DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION SPECTROPHOTOMETRY METHOD FOR SIMULTANEOUS ESTIMATION OF NAPROXEN AND ESOMEPRAZOLE MAGNESIUM TRIHYDRATE IN TABLET DOSAGE FORM

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Accepted Date: 22/04/2012 **Publish Date: 27/04/2012**

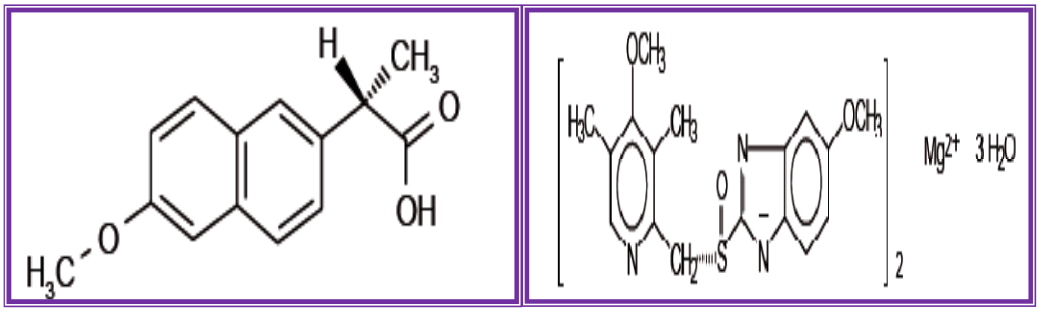
Abstract: A novel, simple, sensitive and rapid spectrophotometric method has been developed for simultaneous estimation of Naproxen and Esomeprazole Magnesium trihydrate. The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths, 276 nm and 302 nm, λ max of Naproxen and Esomeprazole Magnesium trihydrate respectively. Beer's law was obeyed in the concentration range of 10-35 µg/ml and 1-11 µg/ml for Naproxen and Esomeprazole magnesium trihydrate respectively. The mean % recoveries were found to be in the range of 99.26 – 100.35 % and 98.89 – 99.33 % for Naproxen and Esomeprazole Magnesium trihydrate respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of Esomeprazole Magnesium trihydrate and Naproxen in their combined Tablet dosage form.

Keywords: Naproxen, Esomeprazole Magnesium trihydrate, % recoveries, Method validation, Tablet.

INTRODUCTION

Naproxen[1] (NAPRO) is chemically (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid (Figure 1a). It is a drug belongs to a class of NSAIDs (nonsteroidal anti-inflammatory drugs acts by inhibiting isoforms of cyclo-oxygenase 1 and 2). It has an activity to treat inflammatory rheumatoid diseases and relieve acute pain. Esomeprazole Magnesium trihydrate [2] (ESO) is S-isomer of omeprazole and Proton pump inhibitor. It is chemically Di-(S)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2 pyridinyl)methyl]-sulfinyl]-1H-benzimidazole magnesium trihydrate (Figure 1 b).It is used in treatment of peptic ulcer disease, NSAIDS- associated ulceration and Zollinger- Ellison syndrome used as Anti-ulcerative. ESO and NAPRO in combined dosage form (VIMOVO)[3] is used to relieve the signs and symptoms of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis and to decrease the risk of stomach ulcers in patients at risk of developing stomach ulcers from treatment with NSAIDs. The review of literature revealed that various analytical methods involving spectrophotometry [4, 5], HPLC [6,7], HPTLC [8] have been reported for ESO in single form and in combination with other drugs. Several analytical methods have been reported for NAPRO in single form and in combination with other drugs including spectrophotometry [9-11], HPLC [12-15], HPTLC [16].

The present work describes the development of a simple, precise, accurate and reproducible spectrophotometric method for the simultaneous estimation of ESO and NAPRO in their combined dosage forms. The developed method was validated in accordance with ICH Guidelines [17] and successfully employed for the assay of ESO and NAPRO in their combined Tablet dosage form.



(a) (b)

Figure 1 Chemical structure of (a) NAPRO and (b) ESO

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure ESO and NAPRO were kindly provided by Osaka pharmaceuticals, Sakarda, Vadodara. Gujarat, India and Relax Pharmaceuticals, Makarpura, vadodara. Gujarat, India respectively as gift samples. Analytical grade methanol was purchased from RFCL limited, New Delhi, India. Tablet of ESO and NAPRO in combine dosage form, VIMOVO, with a 20 mg ESO and 375 mg NAPRO label claim, manufactured by *AstraZeneca Pharmaceuticals.*

Instruments

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose. All statistical calculations were carried out using Microsoft excel 2010 analytical tool.

Preparation standard stock solutions

Esomeprazole magnesium trihydrate (ESO) standard stock solution (50 µg/ml)

A 100 mg of ESO standard was weighed and transferred to a 100 ml volumetric flask and dissolved in 60 ml methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000µg/ml ESO. From this solution 2.5 ml was transferred to volumetric flask of 50 ml capacity. Volume was made up to the mark to give a solution containing 50µg/ml of ESO.

Naproxen (NAPRO) standard stock solution (100µg/ml)

A 100 mg of NAPRO standard was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 60 ml methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml NAPRO. From this solution 5.0 ml was transferred to volumetric flask of 50 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of NAPRO.

Selection of Analytical Wavelength

1 - 11 µg/ml solutions of ESO were prepared in methanol and spectrum was recorded between 200-400 nm. First derivative spectra for above concentration were obtained. Similarly 10-35 µg/ml solutions of NAPRO were prepared in methanol and spectrum was recorded between 200-400 nm and First derivative spectra were obtained. The overlain derivative spectra of NAPRO and ESO at different concentration were recorded. The Wavelength, for simultaneously detection of both drugs by Simultaneous Equation was 276 and 302 nm selected.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (Coefficient of Variation- C.V.) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. Then absorbance was measured and calculations done to determine the quantity of the drugs.

Linearity

Appropriate volume of aliquot from ESO and NAPRO standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give solutions containing 1-11 µg/ml ESO and 10-35 µg/ml NAPRO. Absorbance at 276 nm and 302 nm were recorded for both drugs (n=6). Calibration curves were constructed by plotting absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

To study the accuracy synthetic powdered mixture was prepared using common excipients in college laboratory and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the placebo at 3 different concentration levels 80, 100, 120 %, taking into consideration percentage purity of added bulk drug samples. Each

concentration was analyzed 3 times and average recoveries were measure.

Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of ESO and NAPRO was carried out by estimating different concentrations of ESO (1, 5, 11 µg/ml) and NAPRO (10, 20, 35 µg/ml), 3 times on the same day and on 3 different days (first, second, fifth)and the results are reported in terms of C.V.

Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10σ/S criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness

The sample solution was prepared and then analyzed with change in the typical analytical conditions like stability of analytical solution.

Reproducibility

The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by another analyst and the values obtained were evaluated using t- test to verify their reproducibility.

Determination of Esomeprazole magnesium trihydrate and Naproxen in their Combined Dosage

Sample preparation (Label Claim: 375 mg NAPRO and 20 mg ESO per tablet)

Powder equivalent to 375 mg NAPRO and 20 mg ESO was accurately weighed and transferred to volumetric flask of 100 ml capacity. Common excipients were also weighed and added. 50 ml of methanol was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with Methanol. The solution was filtered through whatmann filter paper (0.45µ). From this solution 7.5ml was transferred to volumetric flask of 100 ml capacity and volume was made up to the mark with Methanol. From this solution 2.5ml was transferred to volumetric flask of 25 ml capacity and Volume was made up to the mark with

Methanol to give a solution containing 28.125µg/ml NAPRO and 1.5µg/ml ESO.

RESULTS AND DISCUSSION

Simultaneous equation spectrophotometry method was developed for determination of NAPRO and ESO. The proposed method has been extensively validated as per ICH guidelines. Summary of validation parameters for proposed method was given in Table 1.

From overlain spectra of NAPRO and ESO it is clear that NAPRO exhibits λ_{max} at 276 nm and ESO exhibits λ_{max} at 302 nm. The overlain spectra of NAPRO and ESO reveals that the both the drug exhibits distinct λ_{max} and also both drugs shows absorbance at the λ_{max} of each other.so the Wavelength, for simultaneously detection of both drugs by Simultaneous Equation was 276 and 302 nm selected.

Linearity was assessed for ESO and NAPRO by plotting calibration curves of absorbance versus the concentration over the concentration range 1-11 µg/ml and 10-35 µg/ml, respectively.

The following equations for straight line were obtained for NAPRO and ESO.

Linear equation for NAPRO at 276 nm, **Y = 0.017x - 0.056**

Linear equation for NAPRO at 302 nm, **Y = 0.003x - 0.012**

Linear equation for ESO at 276 nm, **Y = 0.056x + 0.041**

Linear equation for ESO at 302 nm, **Y = 0.098x + 0.079**

Simultaneous equation generated:

$C_x = (A_2 \times 0.056 - A_1 \times 0.098) / (0.003 \times 0.056 - 0.017 \times 0.098)$

Where,

1. A_1 and A_2 is absorbance of sample at 276 nm and 302 nm respectively
2. C_x is concentration of NAPRO in µg/ml

$C_y = (A_1 \times 0.003 - A_2 \times 0.017) / (0.003 \times 0.056 - 0.017 \times 0.098)$

Where,

1. A_1 and A_2 is absorbance of sample at 276 nm and 302 nm respectively
2. C_y is concentration of ESO in µg/ml

The % recoveries were found to be in the range of 99.26 – 100.35 % for NAPRO and 98.89 – 99.33 % for ESO (Table 3).The precision of method was determined by repeatability, intraday and interday precision

and was expressed as the C.V. (Table 1), which indicates good method precision.

The Limit of detection for NAPRO and ESO was found to be 1.629 µg/ml and 0.294 µg/ml at 276 nm and 1.584 µg/ml and 0.301 µg/ml respectively. The method was also found to be specific, as there was no interference observed when the drugs were estimated in presence of excipients and robust, as there was no significant change in absorbance up to 48 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to ESO and NAPRO combined tablet dosage form. The results are shown in Table 6.

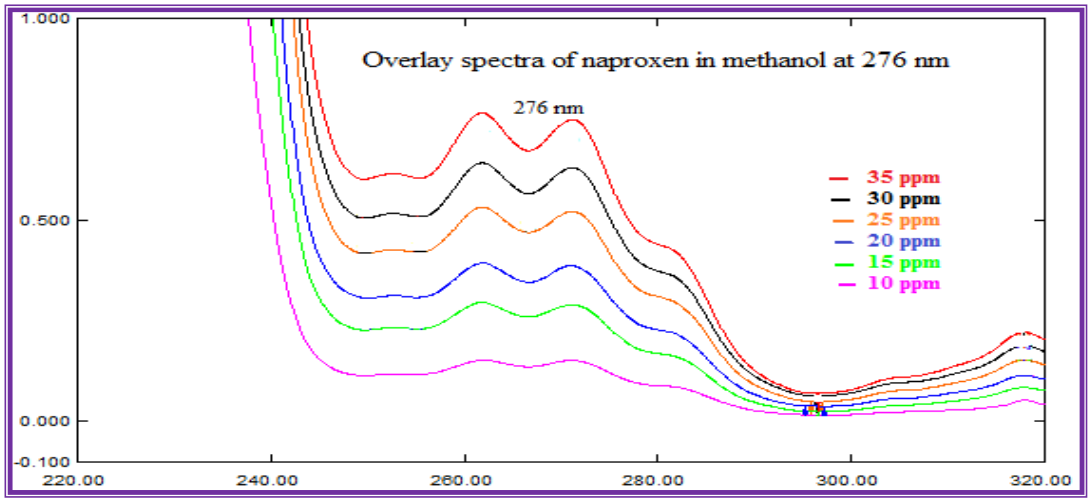


Figure 2 Overlain spectrum of NAPRO in methanol

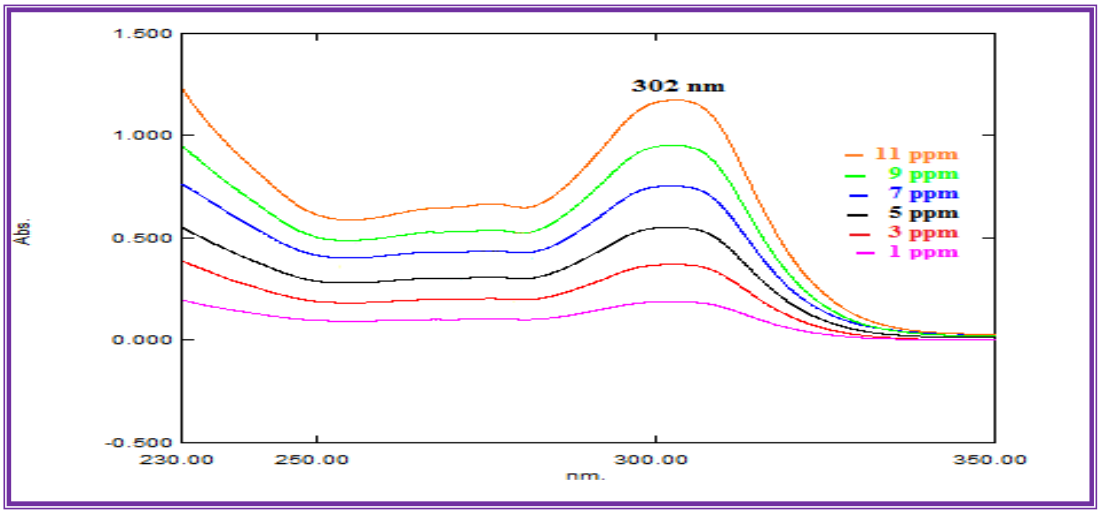


Figure 3 Overlain spectrum of ESO in methanol

CONCLUSION

The proposed first order derivative method provide simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of ESO and ASP in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of ESO and ASP in combined dosage form.

ACKNOWLEDGEMENT

Authors are thankful to Osaka Pharmaceuticals (Vadodara, India) and Relax Pharmaceuticals (vadodara, India) for providing gratis sample Esomeprazole Magnesium trihydrate and Naproxen respectively with the great pleasure. The authors also thankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for providing the necessary facilities for research work.

Table 1

Summary of Validation Parameters of derivative spectrophotometric method

Parameters	NAPRO	ESO
Recovery %	99.26 – 100.35 %	98.89 – 99.33 %
Precision(C.V.) **Repeatability (n=6)** **Intra-day (n=3) Inter-day (n=3)**	0.54698 0.27 – 1.71 0.11 – 1.73	0.93652 0.32 – 0.96 0.28 – 1.47
Limit of Detection (µg/ml)	1.629 at 276 1.584 at 302	0.294 at 276 0.301 at 302
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 48 hrs.	Suitable for 48 hrs.

Table 2

Statistical data for NAPRO by simultaneous equation spectrophotometric method

Parameter	NAPRO at 276 nm	NAPRO at 302 nm
Linear Range (µg/ml)	10-35	10-35
Slope	0.01718	0.00310
Intercept	-0.05582	-0.01198
Standard deviation of slope	0.00035	6.19E-0.5
Standard deviation of intercept	0.00847	0.00149

Table 3

Statistical data for ESO by simultaneous equation spectrophotometric method

Parameter	ESO at 276 nm	ESO at 302 nm
Linear Range (µg/ml)	1-11	1-11
Slope	0.05616	0.09813
Intercept	0.04122	0.07939
Standard deviation of slope	0.00073	0.00130
Standard deviation of intercept	0.00501	0.00894

Table 4

Accuracy data for NAPRO and ESO by derivative spectrophotometric method

% Level	Amount of drug added (µg/ml)		Amount recovered (µg/ml)		% Recovery	
	NAPRO (µg/ml)	ESO (µg/ml)	NAPRO (µg/ml)	ESO (µg/ml)	% NAPRO	% ESO
80 %	22.5	1.2	22.40	1.19	99.56	99.17
100 %	28.125	1.5	28.224	1.49	100.35	99.33
120 %	33.75	1.8	33.5	1.78	99.26	98.89

Table 5

Reproducibility data for NAPRO at 276 nm (28.125 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test*	Inference
0.28067 ± 0.00252	0.21967 ±0.00305	0.225	Not significant difference

* At 95% confidence interval, (t-Tabulated = 4.30)

Table 6

Reproducibility data for NAPRO at 302 nm (28.125 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test*	Inference
0.04833±0.00057	0.04633±0.00115	0.074	Not significant difference

* At 95% confidence interval, (t-Tabulated = 4.30)

Table 7

Reproducibility data for ESO at 276 nm (1.5 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test*	Inference
0.20367±0.00252	0.20200±0.00200	0.038	Not significant difference

Table 8

Reproducibility data for ESO at 302 nm (1.5 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test*	Inference
0.37100±0.00200	0.36767±0.00252	0.009852	Not significant difference

Table 9

Assay Results of Marketed Formulation

Formulation	Actual concentration (µg/ml)		Amount obtained (µg/ml)		% NAPRO±S.D.	% ESO±S.D.
	NAPRO	ESO	NAPRO	ESO		
Tablet	28.125	1.5	28.214	1.493	100.32±0.38	99.56±1.68

n=3 determination

REFERNCES

1. British Pharmacopoeia, London: HMSO Publication 2003, Vol – II: 1303.

2. Indian pharmacopoeia, Govt. of India Ministry of health & family welfare, published by The Indian Pharmacopoeia Commission, Ghaziabad, 2010 (Vol-II):1295-96.

3. "Description and Pharmacology on VIMOVO (Marketed formulation)", July 2011, http://www.druglib.com/druginfo/vimovo/description_pharmacology/

4. Patil SS, Dhabale PN and Kuchekar B: Development and statistical validation of spectrophotometric method for estimation of esomeprazole in tablet dosage form, Asian J. Research Chem 2009; 2:154-156

5. Sharma MC and Sharma S: Spectrophotometric methods for the estimation of esomeprazole magnesium trihydrate in pharmaceutical formulations using indigo carmine reagent. IJPRIF; 3: 1186-90.

6. Armagan Onal and Aysel Oztunc: Development & Validation of HPLC method for the determination of esomeprazole in tablet. Journal of food & drug analysis. 2006; 14: 12-8.

7. Girish GR, Rakesh KS, Pankaj SP, Rajan KS and Brijesh K: RP-HPLC method for the estimation of esomeprazole magnesium in bulk and its pharmaceutical dosage form. IJPHS 2010; 1:125-30.

8. Dalindre HN, Thorve RR, Bugdane PM and Vekariya NR: Validated HPTLC Method For Simultaneous Estimation of Esomeprazole And Domperidone in Tablets 2008; 7.

9. Vinay warmorkar, Pendota santosh, Manjunth SY: Validated spectroscopic method for estimation of naproxen from tablet formulation, Journal of pharmacy research 2011; 4: 2633-2635.

10. Trinath M, Saurabh KB, Hari Hara Teja D and Bonde CG: Development and validation of spectrophotometric method for simultaneous estimation of Sumatriptan and Naproxen sodium in tablet dosage form, Der Pharmacia Sinica 2010; 1:36-41.

11. Gondalia RP and Dharamsi AP: Spectrophotometric simultaneous estimation of sumatriptan succinate and naproxen

sodium in tablet dosage forms, International journal of pharmaceutical biomedical science 2010; 1(2): 24-26.

12. Md.shozan M, Md. Ahsanul H, Md.safiqual I, Ashraful SMI : Development & validation of RP-HPLC method for the simultaneous estimation of Domperidone & Naproxen in tablet dosage form, Journal of Applied Pharmaceutical Science 2011; 01:145-148.

13. Rajnish Kumar, Pinderjit Singh and Harinder Singh: Development & validation of RP-HPLC method for simultaneous estimation of naproxen & pantoprazole in pharmaceutical dosage form. International J. Pharma. Research and Development 2011; 2: 227-232.

14. Tated AG, Khan FA, Gadewar CK and Ghandewar AV: Method Development & Validation for the simultaneous determination of naproxen & pantoprazole sodium in capsule dosage form by RP-HPLC, International J. Pharma. Research and Development 2011; 3: 8-14.

15. Tasnuva H, Md. Mesbah UT, Susmita L, Kanij F, Abul Kalam LK: Development and Validation of RP-HPLC method for simultaneous estimation of naproxen & ranitidine hydrochloride, Pakistan journal pharmaceutical science 2010; 23:379-83.

16. Shubhangini MP, Bharat P and Ravindra YP: Validated HPTLC Method for simultaneous quantitation of Domperidon Maleate and Naproxen Sodium in bulk drug and formulation, Eurasian Journal of Analytical Chemistry 2010; 5:284-292.

17. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: Text and Methodology, Q2 (R1), Geneva, 2005.